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THE BENEFICIAL EFFECTS OF BARIUM CHLORID ON ADAMS-STOKES DISEASE^{*}

REPORT OF THREE CASES

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It frequently happens in the course of complete heart block that attacks of syncope appear associated with standstill of the ventricles. These are the classical attacks known as the Adams-Stokes syndrome. It appears to be a fact that the severity of the attacks depends on the duration of the ventricular asystole. It has been reported¹ that the subcutaneous injection of epinephrin can do away with such attacks, but as the effect of epinephrin generally lasts a very short time, it does not seem likely that the recurrence of attacks is thus prevented. Epinephrin failed to prevent the reappearance of syncope in one of the cases reported here. However, no method has been proposed so far for dealing directly with these attacks in the sense of diminishing their frequency or preventing them. This has been accomplished in the following three cases by the oral administration of barium chlorid.

The periods of standstill are presumably due to the fact that the ventricles, for the moment, contain no mechanism for initiating stimuli. On account of the existence of complete heart block, they can, of course, receive none from the auricles. It occurred to us, therefore, in complete heart block, to make use of the knowledge gained in animal experiments that calcium or barium tended to increase the irritability of the ventricles as indicated by the development of numerous ectopic ventricular beats or ventricular tachycardia.² The same effect has been obtained in the experimental animal as a result of barium chlorid, even when complete heart block is produced by clamping the bundle of His.³ If this were so,

^{*} From the hospital of the Rockefeller Institute for Medical Research and the medical clinic of the Peter Bent Brigham Hospital.

1 Phear, A G, and Parkinson, J. Adrenalin in the Stokes-Adams Syndrome, *Lancet* **1**:933 (May 13) 1922.

2 Rothberger, C J, and Winterberg, H. Ueber die experimentelle Erzeugung extrasystolischer ventrikularer Tachycardie durch Accelerausreizung, *Arch f d ges Physiol* **142** 461, 1911.

3 Van Egmond, A A J. Ueber die Wirkung einiger Arzneimittel beim vollständigen Herzblock, *Arch f d ges Physiol* **154** 39, 1913.

the ventricles, because of the increased irritability, either through some new ectopic focus or by the action of barium on the idioventricular pacemaker, would not permit a long pause of inactivity to occur. In three patients suffering from Adams-Stokes disease, whose histories are here reported, the attempt was made to observe whether an effect comparable to the one observed in experiments might be obtained. Accordingly, we decided to administer barium chlorid, in doses of 30 mg, three or four times a day by mouth.

In considering the efficacy of the use of barium in this condition, it is necessary to compare its action with that of other agents commonly employed to relieve states like this. In Case 1, atropin was injected not only without effect, but, on the contrary, it was followed by aggravation of the patient's condition. In the other two cases, its use was without influence. Digitalis in adequate doses was administered in the first case with the view to improving, in a general way, the circulation of this patient. Mild attacks of the syndrome were already present when the first dose was given. During the second day of treatment, the number and severity of the attacks increased decidedly, so that the conclusion was natural that the effect of digitalis was harmful. In the other two cases, the giving of digitalis was without effect. In Case 1, the inhalation of amyl nitrite gave relief temporarily, in the other two cases, it was not used. Epinephrin was given in Cases 2 and 3. In each instance, it was planned in case of need to give epinephrin, 0.5 c.c. (1:1,000 solution), directly into the heart. This was not necessary in Case 2, since the attacks were of short duration, although frequent intramuscular injections were made. For about one hour after intramuscular injections, the patient would remain free from attacks of syncope, but soon afterward he would be liable again to periods of ventricular standstill. Patient 3 suffered from violent attacks, pauses of the heart lasting from three to four minutes were observed. During such a major attack, an electrocardiogram was taken, which showed that the ventricles fibrillated during a period of about two minutes, followed by a period lasting another minute and a half in which the ventricles failed to give rise to any action detectable in the curves. During these long seizures, 0.5 c.c. of epinephrin was injected directly into the heart, and after a few seconds the heart began to beat.

Since the use of these agents did not prevent the recurrence of the attacks, we resorted to the administration of barium chlorid. The effect of its action was striking in all three cases. The attacks, which had persisted until barium chlorid was given, ceased entirely on the day on which it was started. The effect, in Case 1, was extraordinary. Previous to the administration of barium chlorid, the patient's condition had constantly become worse until, on the day on which it was started, he

resembled patients in the status epilepticus. He was passing in and out of attacks of syncope constantly, being conscious only about one-half the time. The day following the giving of barium chlorid, there were no more attacks or long pauses, although complete heart block persisted. Although barium chlorid was given by mouth in doses of 30 mg three times a day for only two days, he remained free from attacks for some weeks. There was a similar direct relationship between the cessation of attacks and the administration of barium chlorid in the other two cases. All three patients left the hospital free from attacks.

REPORT OF CASES

CASE 1—B. B., a man, aged 55, was admitted in December, 1916, to Rockefeller Hospital, complaining chiefly of pain across the chest and in the head, fainting spells and "hot flashes." There was nothing of significance in the family history. The patient had suffered from measles when a child, but from no other infectious diseases. In the summer months, he had hay-fever. He complained of no symptoms referable to the circulation before the onset of this illness, and appeared to have been in good health.

The illness for which he came to the hospital began in February, 1914, when his complaint was that there were blue spots before his eyes. These would come on with exercise, such as walking. Dizziness occurred at the same time and disappeared on resting. In 1915, he was free of this complaint. In May, 1914, he fell unconscious on the floor and remained so for a short time. A similar attack occurred next day but none after that. In January, 1914, he began to complain of "flashes of heat" over the body. Such attacks recurred at frequent intervals, once a week or oftener, and lasted a few seconds. In July, 1915, he began to complain of precordial pain, which was constant, severe and persistent. In August, 1914, and again in August, 1915, there were attacks of vomiting of two days' duration. For several years there had been cramps of the calves of the legs, which were worse at night, but which did not appear to bear any relation to exercise. His physician stated that he had noticed pulse rates as slow as 9 a minute, and that, about a year before admission, the rate fluctuated between 120 and 24 a minute. At this time, the rhythm was irregular, about 30 a minute.

The patient was not short of breath, at least, in bed. He was pale but not cyanotic. The eyes were prominent. A positive Graefe sign was present. The tonsils were small and atrophic. The superficial lymph nodes, the thyroid gland, lungs and abdomen, except for the liver, were without abnormality. The heart was enlarged, measuring 38 cm in the fourth space on the right side and 14.5 cm on the left side in the sixth space. The point of maximal impulse of the apex was in the fifth space, 13 cm from the middle line. The rate was slow, about 30 a minute. The respirations were irregular, seven long deep breaths being followed by three smaller ones, after which a pause ensued. The liver was not felt, but there was dulness on percussion, to 13.5 cm below the free border of the ribs. The retinal vessels were tortuous. The urine was normal. The Wassermann reaction was negative. There was no edema. It was clear that the patient was suffering from heart block, and this diagnosis was confirmed on examining the electrocardiograms. The auricles were fibrillating. Because of this fact, atropin, 1.2 mg, was injected subcutaneously. The blood pressure (Dec. 22, 1916) before the injection was 240 systolic and 96 diastolic. Afterward, it fell to 200 systolic and 80 diastolic.

On a subsequent occasion (Jan. 4, 1917), atropin, 2.4 mg, was injected subcutaneously. On this occasion, the blood pressure showed no change. After

the injection of atropin the heart rate did not rise. At that time, the blood pressure reading with a Gaertner tonometer was 160. The difference in height between his brachial and tonometer pressures was about 75 mm, so that the brachial pressure was probably approximately 235 mm, a level about average for him. During the course of the atropin test, the "hot flashes" of which he complained on admission reappeared. When these occurred, there were long intermissions in the pulse. January 9, there were many "hot flashes," one very severe, in which the patient became unconscious. Before these "flashes," there was paleness of the face and neck. The eyes became fixed and the pupils wide. The lids then closed for a short time. When they reopened, it was seen that the eyes were rolled quickly upward and to the right, jerking two or three times. There was twitching at the left corner of the mouth. The attack lasted from ten to fifteen seconds. It was not until asystole had persisted for about eight seconds that the rolling of the eyes took place. The patient was given barium chlorid in doses of 40 mg, three times a day for two days (January 12 and 13). On the morning of January 13, the severe attacks of circulatory disturbance ceased and did not recur. The patient left the hospital, February 9, improved.

The difficulty of arriving at an understanding of the nature of the cardiac mechanism in this case was due to the fact that the ventricular rhythm was irregular. It was for this reason, as well as on account of the slow rate, that the atropin tests were given. These injections failed, however, to bring about an increase in the rate of the ventricles. This failure was in all probability not due to inactivity of the atropin because the other signs of atropin action, such as flushing, dryness and dilatation of the pupils were present. Aside from the minor complaints, such as headache, sleeplessness and anorexia, he suffered, between January 1 and 13, from innumerable syncopal attacks, one of which has been described. Some of these were short, others were counted which lasted as long as sixty seconds, and some were even longer than this. Other attacks were short, coming one directly after another, so that the patient gained the impression that he was having one a minute. In any event as many as five or six were counted in a period of ten minutes. The patient usually anticipated the onset of an attack by feeling a sensation of heat, beginning in his feet and gradually mounting upward. In addition, one observed the alternate ashy grayness and flushing of his face. At the time of the attacks and also later there was pain in the precordium.

The remedies that were used have already been enumerated. The effect of barium chlorid has been stated. An analysis of the electrocardiograms was difficult on account of the many forms of the ventricular complexes. There appeared to be at least two distinct foci for stimulus production in the ventricles. After a long pause, it usually happened that the new rhythm was initiated by an ectopic beat (Fig 1). For the most part, after a single initial ectopic beat, a return to the usual form was observed. Occasionally, there was a repetition of this beat. The inference that was drawn was that the usual source of stimulus production in the ventricles underwent what may vaguely be called fatigue. The heart then stood still until the ectopic focus stepped in and averted permanent standstill and death, or within a period not too long the rhythm was taken up at the usual sites. One might expect a difference in rate between the rhythm initiated at the usual and at the ectopic foci, but this was not the case.

After returning to his home, the patient began again to have frequent attacks. The suggestion was then made that he take calcium lactate, 1 gm, three times a day. After this treatment was begun, his physician reported that the attacks ceased, that he had no pain, and that his chief complaint was shortness of breath. The pulse rate was 30. Death was sudden, November 10, when the patient was returning to bed after a bowel movement. No necropsy was obtained.

CASE 2—A S R, a man, aged 32, who first came under observation, Oct 15 1923, gave as the chief complaints, dyspnea on exertion, dizziness and attacks of faintness.

The patient's father and mother were living and well, at the age of 70. He had one sister, aged 43, alive and well. There had been no deaths in the family. His child suffered from asthma, and was sensitive to egg and fish.

The patient had had a severe attack of diphtheria at the age of 4 and was given antitoxin. There was no history of rheumatic fever, chorea, pneumonia, typhoid fever, growing pains, sore throat or tonsillitis. The tonsils had not been operated on. He had always been strong and well. He played a considerable amount of golf.

A year before the patient came under observation, the patient was known to have a normal heart rate. He had been active in athletics, and had been captain of the football team in college. More recently, he had been the local champion golfer. The heart rate, August 7, was about 35. The basal metabolism was -7 per cent. The patient was in good health until nine months before, when he first noticed that he became dyspneic when going up hill and on climbing stairs. Shortly after this, he commenced to have periods of dizziness and, on rare occasions, would lose consciousness for perhaps one or two seconds. These usually occurred after exertion, and before rather than after eating. He became nervous and apprehensive as a result. For many years, he had known that he

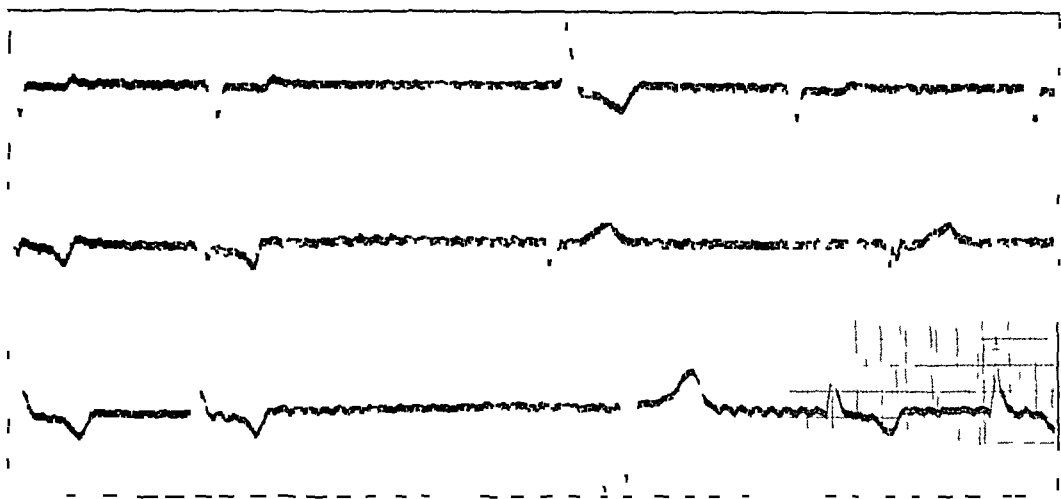


Fig 1 (Case 1) —Complete auriculoventricular dissociation (complete heart block), fibrillation of auricles in Leads I, II and III. At the beginning of each curve are shown two ventricular beats, then follow long periods of quiescence, the longest being in Lead III and measuring 3.32 seconds. The pauses are interrupted in Leads I and III by a single ectopic contraction and in Lead II by two ectopic contractions. The rate of the ectopic beats shown in Lead II is slower than that of the mechanism usual at this time. The dominant ventricular rhythm is irregular. These curves are interpreted as illustrating failure of impulse formation in the ventricles, the circulation being maintained during failure by the assumption of this function by another focus. This conclusion is adopted after the study of many other curves similar to these taken from this patient. The injection of atropin, on several occasions, failed to alter the degree of block by increasing the ventricular rate, that is to say, irregularity is not due to fibrillation of the auricles, which coexisted. Records exist in which periods of quiescence as long as nineteen seconds were photographed. Still longer ones were observed of which no records were made.

was sensitive to fish, and at times reacted to it with attacks of spasmodic asthma, marked by dyspnea and wheezing, urticaria and edema in the throat. Certain kinds of dust also would occasion attacks of asthma. He found that epinephrin usually gave him relief.

The patient looked somewhat sallow. The pupils were dilated, equal, circular, and reacted normally to light. Both tonsils were enlarged and ragged, but showed no exudate. There was no general glandular enlargement. The lungs showed no dullness, bronchial breathing or rales. On palpation and percussion, the heart was slightly enlarged, measuring 1 cm outside the nipple line, 12 cm to the left of the midline. There was no thrill. Auricular beats were definitely heard during the long diastole and, synchronously with these, waves in the veins of the neck were to be seen. The intensity of the first sound at the apex changed occasionally, becoming snapping, muffled or reduplicated. There was a slight systolic murmur at the apex, which was less well heard at the base. There was no diastolic murmur. The heart action was slow and regular, with an occasional lengthening of the diastolic pause. The heart rate was 30 a minute. The blood pressure was 122 systolic and 55 diastolic. The abdomen was negative, except for slight tenderness in the gallbladder region. There was no peripheral edema. The electrocardiograms showed complete heart block with ventricular complexes that indicated defective conduction within the ventricles (Fig 2). The Wassermann reaction was negative.

In October, 1923, a roentgenogram of the heart, taken at a distance of 7 feet (177 cm), showed moderate cardiac enlargement with no abnormalities in the contour. The measurements were as follows: To the right of the medial line, 5 cm; to the left, 9.7 cm, and the great vessels, 5.8 cm. The internal diameter of the chest was 26.3 cm. The roentgenograms of the kidney and gallbladder areas showed the kidneys to be apparently normal in size, shape and position. There was no evidence of calculi.

October 18, the urine was yellow, clear and acid. The specific gravity was 1.015. There was no sugar. There was no albumin. The sediment showed no casts, blood or pus.

October 29, in the phenolsulphonephthalein test, 48 per cent of the drug was excreted in two hours and ten minutes. The vital capacity of the lungs was 4,400 cc. The weight was 75 kg. The height was 5 feet 9½ inches (176.5 cm). The systolic blood pressure was 128 mm of mercury, the diastolic, 60.

During his stay of five weeks at the Peter Bent Brigham Hospital, he had no fever. He gained 26 kg in weight. The fluid intake and output were normal. The bowels were normal. The pulse rate was slow, generally ranging from 20 to 35 a minute. October 16, he was given 1 mg of atropin sulphate subcutaneously, and the heart rate remained constantly at 30 for an hour following the injection. October 17, he was given 2 mg of atropin sulphate, and the heart rate remained constantly at 29 to 30. October 19, 0.5 cc of epinephrin (1:1,000) was given subcutaneously. Following this, the patient felt uneasy and somewhat faint. At this time, the heart rate was 21. During the following hour, the rate rose to 30 and 34.

A few days after his admission to the hospital, long pauses began to interrupt the usual rhythm. At the same time, attacks of dizziness or complete loss of consciousness occurred. Pauses of from five to ten seconds and occasionally of from twelve to fifteen seconds were frequently noted (Fig 3). The patient became very apprehensive, and the situation somewhat critical. It was found that preceding these spells, for a period of about half an hour, the pulse would be 20 or 22, rather than 30, and even with this slow rate, sudden pauses of about four seconds were frequent. If epinephrin (1:1,000) in doses of from 0.3 to 0.5 cc was given subcutaneously, the pulse rate would promptly rise and the dizziness disappear. Five or ten minutes after the injection, the patient would notice that his heart was pounding more rapidly, and he would become slightly nervous and warm. Epinephrin was frequently given, and the administration appeared to prevent impending attacks of syncope, which seemed otherwise to accompany the long pauses of the heart beat. Because there would be slower absorption of the subcutaneous injection during a long

pause of the heart, it was planned to inject epinephrin directly into the heart, if the pause lasted more than thirty seconds. For this purpose, a hypodermic of epinephrin was kept constantly at the bedside.

October 21, the patient had slight convulsions in one of the attacks of unconsciousness. A pause of ten seconds was noted. During the next few days, the patient had frequent attacks, five or ten times a day, which varied in

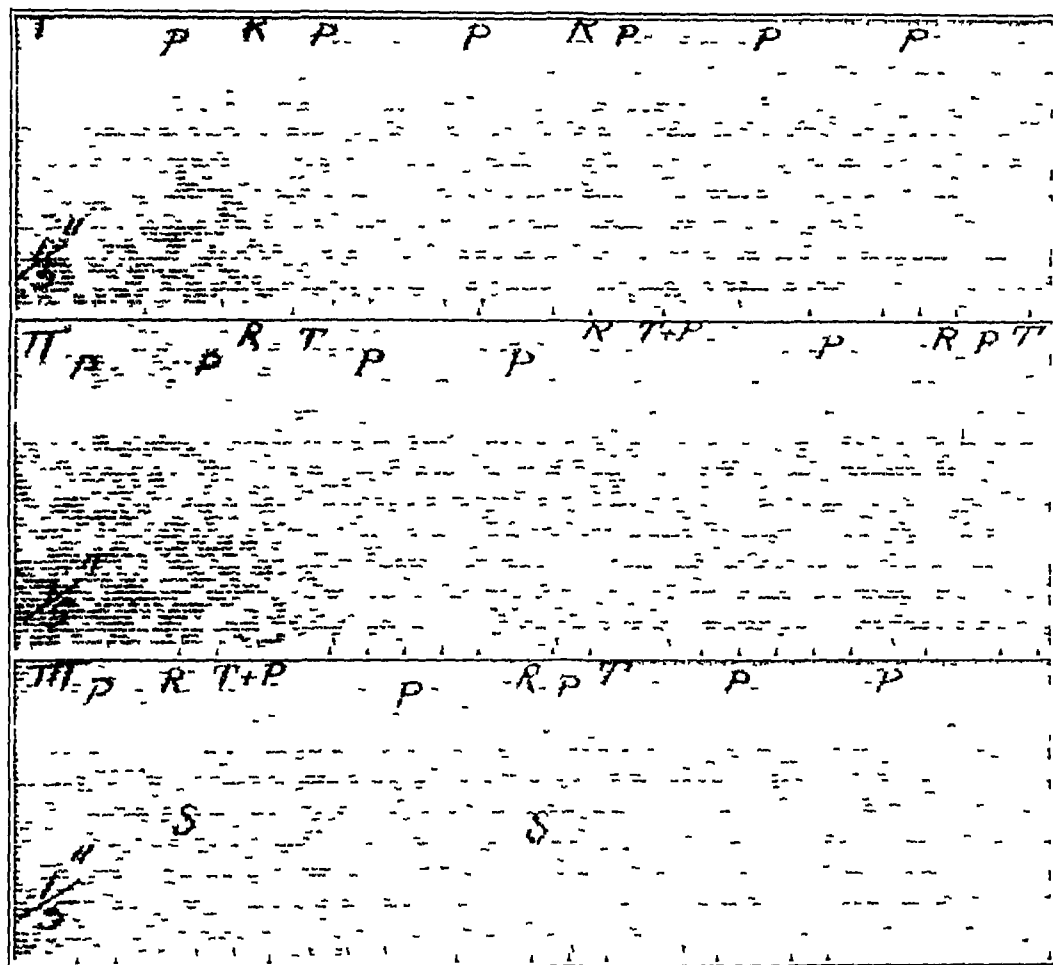


Fig 2 (Case 2)—Oct 15, 1923, complete heart block. In this and in Figure 4 are shown Leads I, II and III. The time is marked below in one-fifth seconds. The auricular rate is 75 and the ventricular, 30.

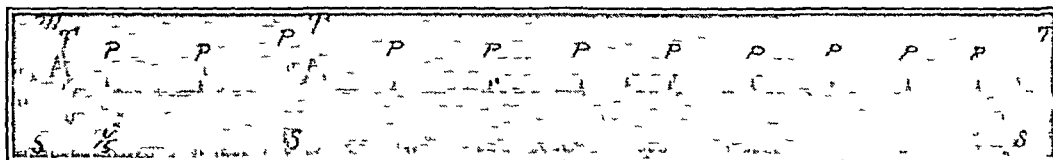


Fig 3 (Case 2)—October 17, Lead III. This curve shows a ventricular pause of about seven seconds, the auricles continue to beat at a rate of about 79.

intensity, the lighter ones being associated with a feeling of faintness, the more severe ones being accompanied by convulsions. He was receiving frequent doses of epinephrin during this time.

October 24, he commenced to take barium chlorid, 30 mg, by mouth, four times a day, followed in half an hour by subcutaneous injections of 0.3 cc of epinephrin. On this day, he had no attack, but he occasionally felt slightly

dizzy This was the first occasion on which he had had no long pauses of the heart beat for the duration of a week The next day, he was again free from attacks, and his general condition improved very strikingly He was propped up in bed, and was able to feed himself Previous to this, he had been afraid to move for fear of precipitating an attack

October 26, the dose of epinephrin was reduced from 0.3 c.c. four times a day to 0.2 c.c. four times a day He had no attacks, October 27 October 28, the dose of barium chlorid was diminished to 15 mg., four times a day, followed in half an hour by 0.2 c.c. of epinephrin He had no attacks on this day or on the 29th

October 30, he had one attack of faintness at 8 10 a. m., during which there was a pause of 6 seconds Epinephrin was given immediately after this attack, but was omitted during the rest of the day He had no attacks for the next two days November 1, he received neither barium nor epinephrin The next day, he was allowed to sit in a chair for fifteen minutes This occasioned him no discomfort He had no attacks on this and the two following days November 5, the effect of an epinephrin nasal spray, 1 1,000, was tried This produced a burning sensation in the nostrils, but no appreciable change in the heart rate

November 6, it was decided to try the effect of full doses of digitalis, and digitalis leaves, 0.1 gm., was given four times a day from November 6 to 12, 2.6 gm. in all were given This did not seem to influence the rate, which remained at about 30 From the time he was allowed to sit in a chair, the patient's activities were greatly increased, and finally he walked around the ward without discomfort He had no further attacks during his stay in the hospital, has remained in excellent health for more than a year since his discharge, and is doing his ordinary work in an insurance business He has taken no barium since leaving the hospital and the complete heart block has persisted

The diagnosis was Adams-Stokes syndrome, complete heart block and chronic myocarditis

It seems rather striking that the attacks of unconsciousness and convulsions ceased abruptly the day he commenced to take barium chlorid, for they occurred frequently up to October 24 From then on, he had only one slight attack Atropin did not relieve the complete heart block Epinephrin always increased the ventricular rate slightly, but this was temporary Full doses of digitalis seemed to have no appreciable effect on the mechanism of the heart beat

CASE 3—A. T. H., a woman, aged 52, a school teacher, who was admitted to the Peter Bent Brigham Hospital, Dec. 31, 1923, complained chiefly of attacks of unconsciousness

There was nothing of significance in the family history

The patient had had scarlet fever at the age of 4 Following this, she had had trouble with the left ear and gradually lost hearing on that side Ten years before, the right ear gradually became deaf She had had diphtheria twice, at the ages of 6 and 7 There was some question as to the presence of paralysis of the leg muscles, at the time of the attacks of diphtheria Following the second attack, she was unable to be up and around for about one year She had had tonsillitis at the age of 22 For about fifteen years, and continuing to four years before, she has experienced, at irregular intervals, frequent attacks of pain in the epigastrium, so severe as to require morphin for their relief The general nature of these attacks resembled gallstone colic

For the last year, the patient has become increasingly short of breath and had had palpitation on exertion One year before admission, she suddenly fainted and fell to the floor She was unconscious for about five minutes Similar attacks had occurred frequently since then She could usually tell a few minutes before when an attack was coming on At times she almost lost consciousness but regained control of herself Her sister, who was a nurse,

had frequently tried to take her pulse during a spell but had been unable to feel it. After an attack, the rate was usually about 30. While she was unconscious, her muscles contracted convulsively. The day before her first admission to the hospital, she lost control of her bowels and bladder during an attack. This was the first time that this had occurred. The attacks might occur as often as twice a day, or there might be periods of two or three weeks in which she was entirely free from them.

The height was 153 cm. The weight was 76 kg. The surface area measured 1.74 sq. m. The patient was a well developed, somewhat obese, middle-aged woman. She lay comfortably in bed in a semirecumbent position. The pupils reacted normally. There was no glandular enlargement. The throat showed no abnormality. The left ear was totally deaf. The patient could hear with the right ear only with the aid of an electrical apparatus. The lungs were clear, there being neither bronchial breathing, dullness or râles. The vital capacity of the lungs was 2,300 cc. January 4, a roentgen-ray examination

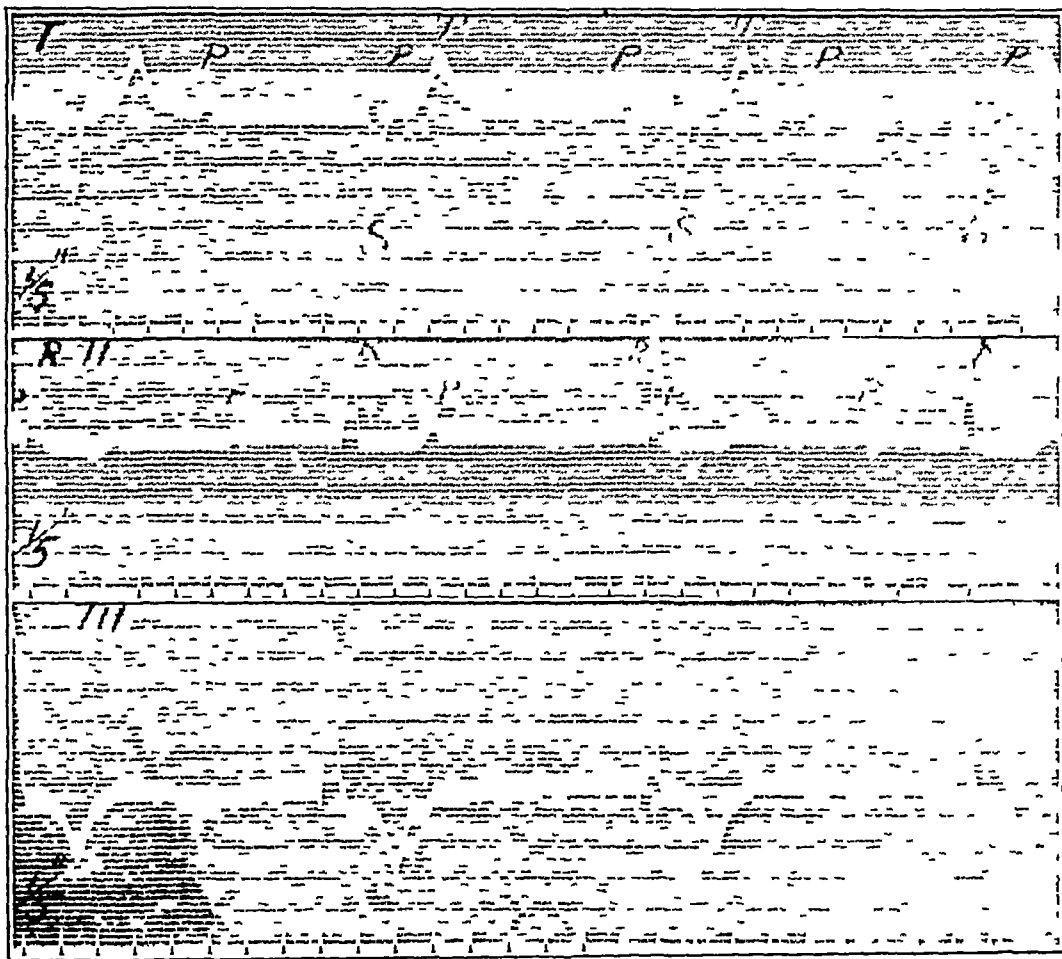


Fig 4 (Case 3)—Jan 23, 1924, complete heart block. The auricular rate is 50 and the ventricular, 35.

of the chest, with the patient in bed, showed marked cardiac enlargement. The lungs appeared mottled. The apex impulse of the heart was seen and felt 13 cm to the left of the midsternal line. The supracardiac dullness in the first space measured 7 cm. There was no thrill at either the apex or the base. The heart rate was slow and irregular, being about 30 a minute. The quality of the first sound changed in different cycles. There was a fairly loud systolic murmur at the apex and a louder and harsher one in the aortic area. There

was no diastolic murmur to be heard. Distinct auricular waves were to be seen in the veins of the neck during the long pauses of the heart. Pressure of the left and right vagus nerves produced no effect on the heart. The Wassermann reaction was negative.

An examination of the stools showed nothing significant. There was no sign of occult bleeding. In the phenolsulphonephthalein test, 55 per cent of the drug was excreted. The urine was clear and acid. The specific gravity was 1.016. There was a slight trace of albumin. There was no sugar. The sediment showed a rare hyaline cast. The hemoglobin was 100 per cent. The red blood cell count was 5,056,000. The white blood cell count was 9,880. A stained smear showed a normal differential count. The erythrocytes appeared normal. The electrocardiograms showed complete heart block (Fig 4).

On the evening of her admission to the hospital, the patient suffered from an attack of unconsciousness at 6 p. m. She gave a slight groan, her arms and legs became stiff, and her breathing ceased. No pulse could be obtained, and no heart sounds could be heard over the precordium for about twenty seconds. Then the heart suddenly began to beat slowly, and almost immediately respiration began. She then turned her head and was able to speak. Jan 1, 1924, she was given 1 mg of atropin subcutaneously. This caused no change in the mechanism of the heart beat. Later the same day, she was given 2 mg of atropin. This dose also was without effect. During the next three days, a total of 19 gm of digitalis leaves was given by mouth which caused no appreciable change in the heart. January 8, a second attack of unconsciousness occurred similar to the first. Following this she became apprehensive and feared another attack, the fear may however have been due to the fact that the heart rate was slower than usual, about 25 a minute.

On the morning of January 13, a third attack occurred. She first turned pale, then uttered a cry and turned her head backward. The eyes were seen to roll up. In a few seconds, she became cyanotic and continued to moan. From the commencement of the attack, no pulse could be felt at the wrist. No heart sounds could be heard over the precordium with a stethoscope. She was immediately given 1 c.c. of epinephrin intramuscularly, and after a standstill for about half a minute, the heart began to contract slowly for 4 or 5 beats, and then gradually accelerated its rate until, in about ten seconds, it was 140. One minute later, the rate was 72, and in another minute, 64. Within three minutes of the onset of the attack, she was conscious again and complained only of exhaustion. The heart then gradually slowed to a rate of 40. There was then coupled rhythm. January 14, a milder attack of unconsciousness occurred. January 15, she commenced to take barium chlorid, 30 mg, by mouth three times a day. Following the administration of this drug, there were no attacks until February 3, when she was discharged from the hospital. January 24, barium chlorid was omitted. In its stead, 5 c.c. of physiologic sodium chlorid solution, three times a day, was administered.

February 3, when the patient was discharged from the hospital, she was advised to continue taking barium chlorid, 30 mg, three times a day. She continued to do this for ten days and felt well, except for an occasional feeling of faintness. She was up and about and able to walk two blocks. Finally, she was persuaded to give up medicine by a Christian science healer. Then she noticed a slow, irregular pounding of the heart. February 23, she had a severe attack and was unconscious for three or four minutes. During the next few days, she had from four to six attacks of unconsciousness each day. The day before her second admission to the hospital, she had six attacks between 2 a. m. and 7 p. m. These were characterized by a large, booming sensation in her left chest, followed by a number of similar "vibrations" of her heart. She was able to call for assistance, and then suddenly was unable to breathe and lost consciousness. She then began to struggle and to twitch all over, often biting

her tongue and becoming incontinent of urine and feces. The spells of unconsciousness lasted from three to five minutes, and when she recovered she had a sensation of water running in her ears and a bitter taste in her mouth. Her sister, who observed these spells, stated that no pulse could be felt for half a minute or more. On recovery, the patient was greatly excited.

The physical examination at the time of the second admission resembled the first. During the first few days, the patient had four attacks of unconsciousness. In two of these, the heart stopped completely for half a minute and this pause was followed by a slow rate, in two, the standstill of the heart was followed by tachycardia with a heart rate of 160. At the time of each attack, 1 c.c. of epinephrin (1:1,000) was given intramuscularly. What epinephrin had to do with the cessation of the attacks is uncertain, for at the time of the injection the patient was unconscious and pulseless. It is doubtful whether absorption could take place under such circumstances. During the succeeding days, between March 8 and 14, the heart beat was frequently in coupled rhythm, and the patient occasionally complained of palpitation. On the evening of March 13, at 10 p.m., a short attack of unconsciousness without convulsions occurred.

On the morning of March 14, she commenced to have another attack. She was immediately given 1 c.c. of epinephrin intramuscularly. For four minutes, between 8:15 and 8:19, no beats were heard at the apex, and no pulse was felt at the wrist. At 8:19 the heart began to beat at a rate of 135. At 8:23 the rate had fallen to 41. The respirations were then 23. The patient's color gradually returned, and she began to regain consciousness at 8:27. At 10:30 another attack was observed, during which she was pulseless and no heart beats could be heard for three minutes. During this spell, there was frothing of the mouth, cyanosis and incontinence of urine. As this attack passed off, there was convulsive straightening of the arms and legs just before the radial pulse became perceptible. Immediately after the standstill of the heart, the rate was 160, seven minutes later, it was 41. On this day, she commenced to take barium chlorid by mouth, 30 mg., four times a day. On the evening of March 4, there were two slight attacks at 8 and 10 p.m. There were no further attacks during her stay in the hospital, and the patient steadily improved until her discharge from the hospital, April 27. Barium chlorid was omitted, March 21.

March 14, during the most violent attacks of complete standstill, epinephrin was administered frequently. During two of the longer spells, 0.5 c.c. was injected directly into the heart. It seemed unlikely that spontaneous contraction would begin for, since the circulation was stationary, it seemed doubtful whether epinephrin injected into the skin would be absorbed. The patient continued to take barium chlorid by mouth for seven days after leaving the hospital, and continued in fair condition. May 28, she died suddenly. Previous to this, she had been taking barium chlorid irregularly, and for several days before her death she took thyroid tablets, 1 grain (0.06 gm.) three times a day.

The diagnosis was Adams-Stokes syndrome, complete heart block and gallstones.

SUMMARY

In three patients with complete heart block suffering from frequent attacks of syncope and convulsions (Adams-Stokes syndrome), the customary therapeutic procedures, including epinephrin, did not prevent the recurrence of attacks, whereas barium chlorid given by mouth, in each instance, promptly proved successful in rendering the

patients free from attacks. The action of barium served to increase the irritability of the ventricles and to prevent the long asystolic periods that occurred in this condition. These successes warrant the trial of barium chlorid when the recurrence of attacks of Adams-Stokes syncope are frequent.

LEUKEMIA

THE RELATIVE VALUES OF CELL MORPHOLOGY AND THE PEROXYDASE REACTION AS DIAGNOSTIC AIDS *

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The demonstration of oxydase and peroxydase ferments¹ in certain cells of bone marrow origin has led to the extensive application of these reactions as diagnostic aids in the differentiation of myeloid and lymphoid leukemia. This is particularly true of the acute leukemias, in which undifferentiated progenitors of the granular and nongranular leukocytes form a conspicuous part of the blood picture.

The popularity of the oxydase reaction is largely due to its supposed ability to distinguish, by simple color reactions, primitive blood cells which, by the ordinary methods of staining, are exceedingly difficult, if not impossible, to differentiate. The diagnostic value of the reaction in a given case naturally depends on its specificity for the cells in question, a point on which there is considerable difference of opinion.

To those hematologists who regard the oxydase reaction as specific for myeloid cells, the presence of these ferments represents a biochemical difference by which they differ from cells of lymphatic origin. Thus, Naegeli,² Schridde,³ and other adherents of the dualistic theory regard it as an important argument favoring the complete separation of the myeloid and lymphoid systems. Schultze,⁴ Peters,⁵ Herxheimer,⁶ Marchand,⁷ Meyer,⁸ and others described oxydases in nongranular "myeloblasts." That lymphocytes may react similarly is denied.

Other observers, however, have failed to see in the oxydase reaction a certain method of cell identification. While the presence of ferments

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1 The nature of the causative elements in the reactions described will not be discussed. For purposes of convenience, they are referred to as ferments.

2 Naegeli, O. *Blutkrankheiten und Blutdiagnostik*, Berlin, 1923.

3 Schridde, H. *Die blutbereitenden Organe*, in Aschoff's *Pathologische Anatomie*, Ed 6, Jena, 2, 1923.

4 Schultze, W. H. *Die Oxydasereaktion an Gewebsschnitten und ihre Bedeutung für die Pathologie, zugleich ein Beitrag zur Differentialdiagnose der Leukämien*, Beitr z path Anat u z allg Path **45** 127, 1909.

5 Peters, Jacob. *Ein Beitrag zur Leukämiefrage*, München med Wchnschr **56** 1478, 1909.

6 Herxheimer, G. *Ueber einen kombinierten Fall von lymphatischer und Myeloblastenleukämie*, Centralbl f allg Path u path Anat **24** 897, 1913.

7 Marchand. *Ueber akute Myeloblastenleukämie*, München med Wchnschr **58** 924, 1911.

8 Meyer, Oskar. *Zwei Fälle akuter Myeloblastenleukämie zugleich ein Beitrag zur Frage der Sog Leukanämie*, Frankfurt Ztschr f Path **15** 40, 1914.

in the granular leukocytes and their immediate precursors (myelocytes) is admitted, their constancy in the still earlier nongranular forms ("myeloblasts" of the dualists and "hemocytoblasts" or "lymphoidocytes" of the unitarians) is questioned. Dunn⁹ and Jochmann and Bluhdorn,¹⁰ among others, described myeloblastic leukemia in which the myeloblasts failed to react. Jolly¹¹ states that "myeloblasts" always react negatively. Rosenthal¹² likewise found that primitive cells contained no oxydase ferments unless granules (specific or azurophilic) were present. Instances in which "myeloblasts" do not contain oxydases are ascribed by the dualists to pathologic loss of the ferments.¹³ The specificity of oxydases is further questioned by Menten,¹⁴ who found them in many cells, including lymphocytes.

Peroxydases are generally regarded as of the same significance as the oxydase ferments, though results obtained by one method cannot be assumed of another. Variations in technical procedures used to demonstrate ferments may partially explain the conflicting results in the literature. That certain methods demonstrate more oxydase containing cells than others has been frequently noted.

The data presented in this paper were obtained by the use of a benzidin peroxydase stain¹⁵ and controlled by duplicate smears prepared with Jenner-Giemsa or Wright's stains. The peroxydase solution is of the following composition: 80 per cent methyl alcohol, 25.0, benzidin, C P, 0.1, and hydrogen peroxid, 2 drops.

The solution is poured over the blood smears and, after a minute, is diluted with an equal amount of distilled water. This is allowed to remain for five minutes, after which it is poured off, the slide washed with distilled water, and counterstained by Jenner-Giemsa or Wright's method. There is a distinct advantage in counterstaining with the same stain used in ordinary differential counts, as all structures that do not contain peroxydases are stained as usual, and may be easily identified.

With this method, I have, in a routine manner, performed the peroxydase reaction in a series of leukemic and nonleukemic condi-

9 Dunn, J. S. The Use of the Oxydase Reaction in the Differentiation of Acute Leukaemias, *Quart J Med* **6** 293, 1913.

10 Jochmann and Bluhdorn. Ueber akute Myeloblastenleukämie, *Folia haematol* **12** 181, 1911.

11 Jolly, J. Traite technique d'hématologie, morphologie, histogenese, histophysiologie, histopathologie, Paris, 1923, pp 379 and 955.

12 Rosenthal, N. Studies on the Oxydase Reaction of the Cells in Normal and Leukemic Blood, *Arch Int Med* **20** 184 (Aug.) 1917.

13 Von Jagić and Neukirch. Ueber das Auftreten grosser mononuclearer ungranulierter Zellen im Blute chronischer Myelamien, *Berl klin Wchnschr* **47** 874, 1910.

14 Menten, Maud L. A Study of the Oxydase Reaction with Alpha-Naphthol and Paraphenylendiamin, *J M Res* **40** 433 (Sept.) 1919.

15 McJunkin, F. A. A Benzidin-Polychrome Stain for Blood, *J A M A* **74** 17 (Jan 3) 1920.

tions, and have obtained results that are at variance with the prevalent opinion regarding its value as a diagnostic procedure

STAINING REACTIONS OF LEUKOCYTE GRANULES

In slides stained by the peroxydase method, a positive reaction is indicated by the presence of brownish or bluish granules in the cytoplasm of the cells. If granules that do not react positively are present, they may be demonstrated by suitable counterstains.

The relation of ferments to the cytoplasmic granules is disputed. Naegeli and many others believe that certain nongranular cells react positively. According to Pappenheim,¹⁶ the reacting substances are in the cytoplasm, but not in the granules. The reaction appears, however, to be confined to the cytoplasmic granules, conforming to the latter in size and shape. Pantón, Tidy and Pearson¹⁷ and Rosenthal¹² have likewise considered the granules as the bearers of the reacting substances.

In normal blood, the neutrophil and eosinophil granules react positively for peroxydases, the latter more rapidly and strongly than the former. Those monocytes which contain azurophilic granules also react, though less intensely. The statement of Naegeli that monocytes react with the same intensity as the neutrophils is not confirmed. Rosenthal found considerable differences in the reactions of monocytes. Sabrazès¹⁸ found that monocytes with round nuclei contained no ferments, while those with lobulated nuclei reacted positively. Pantón, Tidy and Pearson found them to react only when azurophilic granules were present.

If the preparation is counterstained, it is observed that the basophilic granules are stained thereby, but that they contain no peroxydase. Graham's¹⁹ results were similar, though Naegeli² and Pappenheim¹⁶ describe positive reactions in basophils. Dunn,²⁰ using an oxydase method, found the basophils to react less strongly than the granules of other cells. Rosenthal found them to react for oxydases, but not for peroxydases.

No ferments were demonstrated in the azure granules of lymphocytes or of blood platelets.

Peroxydases were never present in nuclei, nor were they found in the basophilic substances (polychromasia and basophilic stippling) of erythrocytes.

16 Pappenheim, A. *Morphologische Hamatologie*, Leipzig, **2**, 1919.

17 Pantón, Tidy and Pearson. *The Leukaemias. An Analysis of Fifty-Nine Consecutive Cases*, Quart. J. M. **7** 340, 1914.

18 Sabrazès, J. *Des oxydases et peroxydases du sang*, Arch. d. mal. du coeur **15** 841, 1922.

19 Graham, G. S. *The Hemic Basophil*, J. Exper. Med. **31** 209 (Feb.) 1920.

20 Dunn, J. S. *The Oxydase Reaction in Myeloid Tissues*, J. Path. & Bacteriol. **15** 20, 1911.

In leukemic blood, the immature cells contain types of granules other than those already mentioned

Certain of the immature cells contain granules stainable by azure-eosin mixtures, that differ from the azure granules found in normal lymphocytes and monocytes. Theories regarding the significance of these granules have led to considerable discussion

Naegeli describes them as "unripe" neutrophilic granules, and terms the cell that contains them "promyelocyte." Their nongranular predecessor is the "myeloblast."

Ferrata²¹ believes that they indicate an early stage in myeloid differentiation. He recognizes two varieties, a small type indicating the first stage of neutrophil granulogenesis ("proneutrophil" granules), and a larger type as the forerunners of the eosinophils ("proeosinophil" granules). These granules later disappear, their place being taken by the specific granulation. In Ferrata's terminology, these cells are the "myeloblasts" (proneutrophilic or proeosinophilic), which are derived from the nongranular "hemocytoblast." The "myeloblast" of Naegeli is therefore morphologically identical with the "hemocytoblast" of Ferrata, the "promyelocyte" or Naegeli, with the "myeloblast" of Ferrata.

According to Pappenheim¹⁶ and Jolly,²² these azure granules are of no diagnostic import, are inconstant in their appearance, and may be present in either myeloid or lymphoid cells.

When the peroxidase reaction is applied to cells containing these granules, they react positively. This is amply confirmed by control specimens in which the percentage of cells is calculated as indicated in the method given below. Furthermore, the counterstain fails to demonstrate any granules that do not react, though the azure granules of lymphocytes are clearly stained. It is necessary, of course, to use a counterstain containing azure and eosin, such as Wright or Giemsa stain. In azurophilic bodies of abnormal form (such as the rod-shaped Auer bodies), the ferment is confined to the rod itself.²³ These rods probably occur only in myeloblasts in acute myelogenous leukemia.

It seems logical to conclude, therefore, that the azurophilic granules of immature cells indicate differentiation toward myelocytes. I have therefore followed Ferrata's nomenclature in classifying them and refer to them as "myeloblasts."

21 Ferrata, A. Einige neue Feststellungen über die Vorstufen der Granulozyten, *Folia haematol.* 9: 549, 1910; *Le Emopatie*, Milan, 1, 1918.

22 Jolly (Footnote 11, pp 222 and 977).

23 Rosenthal (Footnote 11). Ishikawa, T. Ueber den Auerkörper bei einem Falle von akuter Myeloblastenleukämie, *Mitt. a. d. med. Fakult. d. k. Univ. zu Tokyo*, 1919; Richter, M. N. The Presence of Auer Bodies in Leukemic Tissues, *Arch. Int. Med.* 31: 677 (May) 1923.

As noted above, Ferrata described a large type of granule as the forerunner of the eosinophil. This variety occurred so infrequently that no conclusions regarding its nature or peroxylase reaction can be drawn here. Many eosinophil myelocytes, however, contained varying numbers of basophil granules. These differed from Ferrata's "proeosinophil" granulation in being basophilic rather than azurophilic. Graham¹⁹ considers these as degenerate eosinophils. Downey,²⁴ however, regards them as immature eosinophil granules. Jordan and Speidel²⁵ have found them in lower forms, and conclude that they represent a phylogenetically and ontogenetically early stage of eosinophil granulogenesis.

With the peroxylase solution, these granules fail to react, though the eosinophil granules of the same cell react strongly. They are the only negatively reacting granules that were found in cells containing peroxylases.

It is possible that the monocytes also have an immature form of granulation. In a case of acute myelogenous leukemia with associated anemia of the primary type (so-called leukanemia), I have observed immature monocytes (monoblasts) with azure granules slightly larger than normal, which failed to react for peroxylases.

In the series of cases here reported, the nongranular primitive cells (hemocytoblasts) have always reacted negatively.

PEROXYDASE PERCENTAGES IN LEUKEMIA

The data concerning the peroxylase reaction of leukocytic granules reported above may be confirmed by comparing the percentage of peroxylase containing cells with the sum of the percentages of those cells which contain granules of the types thought to react positively, as determined from control specimens prepared by suitable methods. It will be found that, in good preparations, the calculated and observed results agree within the limits of error in counting. In the present series, cover slip preparations were used exclusively, as it is believed that the distribution of cells is more uniform, especially in cases with low total leukocyte counts. The stains used were either Wright or Jenner-Giemsa.²⁶

24 Downey, Hal. Heteroplastic Development of Eosinophil Leukocytes and of Hematogenous Mast Cells in Bone Marrow of Guinea-Pigs, *Anat. Record* **8** 135, 1914.

25 Jordan, H. E., and Speidel, C. C. Studies on Lymphocytes, III, Granulocytogenesis in the Salamander, with Special Reference to the Monophyletic Theory of Blood Cell Origin, *Am. J. Anat.* **33** 485 (July) 1924.

26 The smear should be covered with Jenner's stain for three minutes, then an equal amount of distilled water should be added and let stand for one minute. This should be poured off and, no washing being done, diluted Giemsa stain (1 drop to 1 c.c. of distilled water) should be added and let stain for from ten to fifteen minutes. The smear should then be washed in distilled water, dried, and mounted in neutral balsam or euparal.

The importance of using a good polychrome stain will be discussed later

Leukemic blood varies considerably in the relative proportions of undifferentiated and partially differentiated cells which it contains. As a rule, the chronic leukemias contain a relatively large number of the latter type, while the acute leukemias contain large proportions of undifferentiated cells (hemocytoblasts)

As the hemocytoblasts contain no peroxidases, while these ferments are present in granular myelocytes, the percentage of reacting cells in cases of myeloid leukemia depends on the relative number of each type which is a very variable quantity

The examples of actual counts recorded in the accompanying tables are of interest in that they illustrate the close agreement of the calculated and observed results of the peroxidase reaction. The terminology used in classifying the cells is that of Ferrata

TABLE 1—*Chronic Myeloid Leukemia*

	Leukocytes, 150,000 Per Cent
Hemocytoblasts	33
Promyelocytes, neutrophilic	3
Promyelocytes, eosinophilic	1
Myelocytes, neutrophilic	2
Polymorphonuclear neutrophils	33
Lymphocytes	23
Rieder cells (with azurophil granules)	5
Megakaryocytes	2
Peroxydase percentage	calculated, 44%, observed, 42%

The differential count presented in Table 1 from a case of chronic myelogenous leukemia, contains a variety of mature and immature cells of which all except the hemocytoblasts, lymphocytes and megakaryocytes contained granules of the peroxidase type. Adding their percentages, we would expect to find 44 per cent positively reacting cells. Actual count showed 42 per cent.

The course of this case was followed for several months, during which time the blood count varied considerably. At a later date, the differential count was that shown in Table 2.

The principal change is in the reduction in the number of the hemocytoblasts. The myeloblasts in this classification contain azurophil granules, and react positively. The hemohistioblasts were undifferentiated, and contained no granules. It has been shown that the peroxidase reaction in hemohistioblasts is negative unless granules are present.²⁷

²⁷ Richter, M. N. Observations on the Hemohistioblast of Ferrata. *Am J M Sc* 169 336 (March) 1925

From the count in Table 2, we would expect to find 59 per cent reacting cells. Actual count showed 61 per cent.

The case of acute myelogenous leukemia presented in Table 3 is of interest in that it contains a large percentage of negatively reacting cells (mainly hemocytoblasts). The plasma cells, which appear in small numbers in many conditions, are usually nongranular as they appear in the blood stream, though they may contain cytoplasmic granules in the

TABLE 2—*Chronic Myeloid Leukemia*

	Leukocytes, 110,000 Per Cent
Hemohistioblasts	2
Hemocytoblasts	9
Myeloblasts	14
Promyelocytes, neutrophilic	5
Myelocytes, neutrophilic	9
Metamyelocytes	18
Polymorphonuclear neutrophils	9
Lymphocytes	18
Monocytes (with granules)	4
Monocytes (without granules)	9
Rieder cells (without granules)	3
Peroxydase percentage calculated, 59%, observed, 61%	

TABLE 3—*Acute Myelogenous Leukemia*

	Leukocytes, 63,800 Per Cent
Hemocytoblasts	75.0
Myeloblasts	0.5
Promyelocytes, neutrophilic	2.5
Myelocytes, neutrophilic	1.0
Myelocytes, eosinophilic	0.5
Metamyelocytes	3.0
Polymorphonuclear neutrophils	1.0
Lymphocytes	12.5
Monocytes (with granules)	1.5
Rieder cells (with granules)	2.0
Plasma cells	0.5
Peroxydase percentage calculated, 12%, observed, 13%	

tissues.²⁸ The writer has shown²⁹ that one variety of granule (Russell's fuchsin body) gives a positive peroxydase reaction.

COMMENT

The clinical value of the peroxydase reaction depends on its specificity and the degree of accuracy with which positively or negatively reacting cells may be identified.

28 Dubreuil, G., and Favre, M. Cellules plasmatiques, Plasmazellen à granulations spécifiques, Cellules à corps de Russell (Cytologie et formes évolutives), Arch d'anat micr **17** 302, 1920-1921.

29 Richter (Footnote 23, third reference).

As indicated in the foregoing observations, a positive reaction indicates the presence of cells of the granulocytic series,³⁰ while a negative reaction does not necessarily indicate their absence

Many of the early workers on oxydases reported them in non-granular cells that were thought to be of myeloid origin ("myeloblasts" of Naegeli) Probably some of the confusion is due to variations in technic As has been noted, different results are obtained by varying the methods of demonstrating ferments There is, however, another source of error, the variations in the staining technic of the control specimens

Thus, Dunn, using Jenner's stain as a control, reported that, in a fetus of three and one-half months gestation, a certain percentage of the nongranular cells reacted positively for oxydases He concludes that "these cells were without doubt the nongranular mother cells of the myelocytes, and it was evident that in them the appearance of the oxydases had preceded recognizable granule formation" Schultze⁴ reported oxydases in cells that contained no granules, by Ehrlich's staining method Neither Jenner's nor Ehrlich's solutions contain methylene azure, which is essential for the staining of the azurophilic granules The conclusions of Dunn and of Schultze that the cells in question were nongranular may therefore be questioned, as their methods were inadequate for granule demonstration

At a later date, Dunn⁹ observed that the oxydase reaction is negative in the more early forms of marrow cells, and that, when positive, it is associated with changes that are recognizable by the ordinary methods of staining

Panton, Tidy and Pearson¹⁷ controlled their preparations with Leishman's stain, which demonstrates azure granules, and concluded that the nongranular "myeloblasts" do not contain oxydases Rosenthal used Jenner-Giemsa stain and obtained the same results

Chosrojeff³¹ reported a case of myeloblastic leukemia in which great reliance was placed on the results of the oxydase reaction As many of the "pathologic lymphocytes" (myeloblasts) contained the typical myeloid azurophil granulations, the positive oxydase reaction should be expected According to my view regarding the significance of these granules, the correct diagnosis could have been made from morphologic studies alone

30 Myeloblast to granulocyte The reaction indicates cell type, but not necessarily the tissue of cell origin The significance of peroxydases in monocytes is uncertain, as these cells probably arise in more than one tissue

31 Chosrojeff, G P Myelosis Aleucaemica Acuta Micromyeloblastica, *Folia haematol* 20 33, 1915

Fiessinger and Roudowska³² reported positive oxydase reactions in nongranular cells, but, at a later date, Fiessinger and Broussolle³³ found them to react negatively

In the cases of Marchand,⁷ Meyer⁸ and others, the staining technic for the controls is not given

As it is necessary to employ proper technic for staining control smears, this is even more important in controlling sections of organs Kwasniewski,³⁴ for example, found that the results of the oxydase reaction on blood smears did not always correspond to the results obtained on sections in the same case It is exceedingly difficult, frequently impossible, to establish that cells in the control tissue sections contain no granules It is usually better to use, as controls, tissue smears or imprints stained by a suitable method³⁵

Cases comparable to those of other writers, in which peroxydase containing cells were devoid of all granules in suitably stained controls were not encountered In the cases observed by me, the peroxydase reaction has yielded no information that could not be obtained from well prepared blood smears stained by a suitable polychrome method

To be sure, cases of chronic myelogenous leukemia have a high percentage of reacting cells, but chronic myelogenous leukemia is sufficiently easy of diagnosis from ordinary slides It is in the acute leukemias, in which the primitive nongranular forms predominate (sometimes forming more than 90 per cent of the total leukocytes), that the peroxydase reaction is resorted to, and, in my experience, it is in just these cases that it fails to add data of diagnostic importance

Careful examination of the preparations reveals the fact that the nongranular elements of both myelogenous and lymphatic leukemia in both chronic and acute forms, react negatively for peroxydases The percentage of these cells may be counted in the counterstained peroxydase preparations just as accurately as in the controls, as they appear the same in both If, therefore, these elements constitute a considerable

32 Fiessinger and Roudowska La reaction microchimique des oxydases dans les tissus humains, Arch de med exper et d'anat path **24** 585, 1912

33 Fiessinger, N, and Broussolle, J Etude biologique de la cellule indifferenciee des lucemies aigues, Bull et mem Soc med d hôp Paris **45** 211 (Feb 18) 1921

34 Kwasniewski Ein Beitrag zur Klinik und Histogenese der akuten Myeloblastenleukamie, Deutsch Arch f klin Med **145** 83, 1924

35 Ferrata's modification of the Jenner-Giemsa method for tissue smears is as follows The smears should be dried in air for at least ten minutes, then covered with Jenner's stain for from eight to ten minutes The Jenner's stain should be diluted with twice its volume of distilled water for three minutes This should be poured off and diluted Giemsa stain (2 drops to 1 c.c. of distilled water) should be added and let stand for at least fifteen minutes The smear should then be washed in distilled water, dried and mounted in neutral balsam

proportion of the total, their negative reaction may lead to an erroneous diagnosis of lymphatic leukemia

These observations admit of wider biologic application. The question concerning the identity or nonidentity of the stem cells of the myeloid and lymphoid tissues has long engaged the attention of hematologists. As reviews of the principal theories have recently been given by Fineman³⁶ and by Downey,³⁷ the subject will not be discussed in detail. Suffice it to state that, in their reactions to the peroxydase solution, the primitive elements ("myeloblasts" and "lymphoblasts" of Naegeli) cannot be distinguished one from the other. Nor has it been possible, in the control preparations, to discern morphologic criteria by which they can be distinguished. It seems, therefore, that these cells are alike morphologically, and biologically as far as is indicated by their similar (negative) peroxydase reaction and their potential capacity for differentiation into either myeloid or lymphoid cells. The ferments are attributes acquired only in the process of differentiation along certain lines.

It appears inadvisable to denote this blood stem cell by either of the terms "myeloblast" or "lymphoblast," as they imply specificity that has not been proved to exist. In the counts that have been recorded, this cell is denoted by the term "hemocytoblast."

As the hemocytoblasts in both myelogenous and lymphatic leukemia contain no peroxydases, the diagnosis in cases in which they predominate cannot be made from the peroxydase reaction. However, it may usually (but not always) be made by careful morphologic study to determine the direction of differentiation of the hemocytoblast. Thus, in the case of acute myelogenous leukemia (Table 3) containing 75 per cent hemocytoblasts, the diagnosis was made by the distribution of the other 25 per cent. As the latter included all stages of myeloid differentiation (myeloblasts, promyelocytes, myelocytes, metamyelocytes to polymorphonuclears), the diagnosis of myeloid leukemia was made (confirmed at necropsy), had it included stages in the transition from hemocytoblasts to lymphocytes, with absence of myeloid differentiation, the diagnosis of lymphatic leukemia would have been made. Undoubtedly, cases occur in which blood studies alone are insufficient for diagnosis, though these are exceptional. The frequent presence of myelocytes in lymphatic leukemia seldom causes difficulty, as all transition stages are not usually present.

³⁶ Fineman, S. A Study of Microlymphoidocytic Leukemia, with the Report of a Case, *Arch Int Med* **29** 168 (Feb) 1922.

³⁷ Downey, Hal. The Occurrence and Significance of the "Myeloblast" Under Normal and Pathologic Conditions, Preliminary Account, *Arch Int Med* **33** 301 (March) 1924.

In those cases of hemocytoblastic leukemia³⁸ in which the hemocytoblasts fail to differentiate in either direction, the differential diagnosis of the tissue involved is not made from the blood. The leukemic cells, in these cases, are the mother cells of both myeloid and lymphoid tissues, and may arise in either

SUMMARY

1 Leukocytic peroxydases demonstrable in blood smears by the benzidin method herein described occur only in those leukocytes which contain granules of one of the following types: (a) neutrophilic, (b) eosinophilic, (c) azurophilic granules of monocytes, and (d) azurophilic granules of the myeloblasts of Ferrata (promyelocytes of Naegeli), including Auer bodies.

2 Leukocytes totally devoid of granules do not react for peroxydases.

3 Elements containing the following types of granules do not react: (a) basophilic, (b) basophilic (immature) granules of eosinophil myelocytes, (c) azure granules of lymphocytes, and (d) granules of platelets and megakaryocytes.

4 The peroxydase reaction is of no value in differentiating hemocytoblasts in cases of myeloid leukemia from those of lymphoid leukemia.

5 The azurophilic granules of myeloid cells (myeloblasts of Ferrata) probably represent an early stage of myeloid transformation of the hemocytoblast.

6 The peroxydase reaction is of less value in diagnosis than the study of stained smears.³⁹

38 Ferrata, A. *Le Emopatie*, Milan, 2, 1923.

39 Since this paper was submitted for publication, Piney (*J. Path. & Bacteriol.* 28: 97 [Jan.] 1925) has reported observations on the oxydase reaction in three cases of acute myelogenous leukemia. Using the indophenol reaction controlled by a modified Kardos staining solution, Piney found that "a completely nongranular precursor of the granular leukocytes exists but does not give a positive oxydase reaction, which appears only in those cells in which granules are visible with suitable staining and illumination." The nongranular cells to which Piney refers as "myeloblasts" correspond to the hemocytoblasts in the terminology used in this paper. As shown above, they also react negatively for peroxydases.

BLOOD VOLUME

VI THE RELATIONSHIP BETWEEN BLOOD VOLUME, TOTAL CORPUSCLE CONTENT AND ALKALINE RESERVE IN CASES OF PERNICIOUS ANEMIA *

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The work reported here was done in 1919. In view of the then prevailing association of a much reduced blood volume with pernicious anemia and the reported decrease in alkalinity of the serum, it seemed within the bounds of probability that if the total carbon dioxide elimination had to be taken care of by a reduced amount of blood, the level of the carbon dioxide in each unit of blood would be raised, and that the increase in hydrogen concentration resulting might in turn tend to maintain a reduced blood volume.

I therefore attempted to see to what such an hypothesis would lead by a study of the effect on blood volume of a decrease in the hydrogen ion concentration of the blood brought about by administration of carbonates to two patients with pernicious anemia who had received transfusions of unlike group blood. Changes in the concentration of the unagglutinable corpuscles were used as an index of change in volume of the blood.

The protocol of one of these cases, Case 59, has been given in detail elsewhere,¹ and will simply be reviewed here. An obese woman with an extremely low blood volume, as indicated by the count of unagglutinable corpuscles, began to take bicarbonate. Two days later, the blood volume was apparently greatly increased, seven days later, the count of the unagglutinable corpuscles indicated the normal blood volume proportion of 82 cc for each kilogram of body weight, which remained constant, but as at this time the transfused blood had been in the patient's circulation for thirty-three days, it was questionable whether this drop in the unagglutinable corpuscles indicated a dilution due to increase in blood volume, or one of these intermittent periods of elimination by which the transfused blood is removed from the circulation, which happened to be coincident with the initiation of the administration of the bicarbonate.

In a second case, Case 61 (Table 1), after three transfusions the patient began to take sodium bicarbonate, beginning with the small dose of 0.67 gm a day, and gradually increasing the dose. The apparent blood volume, as indicated by the transfused corpuscles derived from the

* From the Mayo Foundation

1 Ashby, Winifred. Study of Blood Volume, V, The Effect of Treatment on the Blood Volume of Patients with Pernicious Anemia, Arch Int Med **35** 733 (June) 1925

unlike group transfusion given seventeen days before, was 66 c c for each kilogram of body weight one day after administration of the bicarbonate was instituted. An apparent gradual increase in blood volume followed, until, ten days later, the decrease in the count of unagglutinable corpuscles indicated a dilution such that the volume was 111 c c for each kilogram of body weight. At this time, a fresh transfusion of unlike group blood was given. The data derived from it indicated a blood volume of 109 c c for each kilogram of body weight. This close approximation between the volume obtained on the injection of fresh unagglutinable corpuscles and that indicated by the count of the transfused corpuscles that had been in the circulation for twenty-

TABLE 1—*Findings in Case 61*

A man, aged 42, with pernicious anemia, who was very weak, received his first series of transfusions, which did not greatly improve his condition. He died shortly after his return home.

Days of Treatment	Red Cell Count in Millions	Corpuscles for Each Kilogram of Body Weight	C c of Blood for Each Kilogram of Body Weight	Remarks
Transfusion of unlike group blood				
0	1.70	95×10^9	56	Throbbing sensations
1	1.77	106×10^9	60	Throbbing sensations
3	1.88	117×10^9	62	Throbbing sensations
6	1.57	102×10^9	65	Throbbing ceased
9	Transfusion			
14	2.03	107×10^9	51	
14	Transfusion			
15	2.25	115×10^9	51	
16	Patient began taking sodium bicarbonate, 0.67 gm a day			
17	2.16	143×10^9	66	
19	1.94	134×10^9	69	Patient said that he felt better and had an appetite for the first time
20	2.30	159×10^9	69	
24	2.05	152×10^9	74	Patient felt better
27	1.56	173×10^9	111	
Transfusion of unlike group blood				
27	2.12	231×10^9	109	
28	2.57	194×10^9	74	Patient had discontinued bicarbonate while in hospital for transfusion
29	2.76	284×10^9	103	Patient had taken 4 gm of bicarbonate, urine acid
30	2.33	254×10^9	109	
31	2.57	257×10^9	100	Patient had taken 7.33 gm bicarbonate, urine acid

seven days would reduce the probability that the apparent increase in blood volume had been due to elimination of the transfused corpuscles rather than to dilution from an increased blood volume. However, it is not absolute evidence.² While the patient was in the hospital for this transfusion, he took no sodium bicarbonate. The following day, the concentration of the unagglutinable corpuscles was such as to indicate a decrease in blood volume to 74 c c for each kilogram of body weight.

2 Ashby, Winifred. Study of Blood Volume, III, Apparent Changes in Blood Volume Indicated by Transfusion, and Their Bearing on Methods of Determining Blood Volume by Means of the Degree of Change in a Constituent of the Blood, Following Transfusion of a Known Amount of That Constituent, Arch Int Med **35** 641 (May) 1925.

Administration of bicarbonate was resumed, in larger amounts, and after a day a blood volume of 103 c c for each kilogram of body weight was indicated by the count of the transfused unlike group blood corpuscles. This was maintained for the next two days, while the patient was under observation, during which bicarbonate was taken

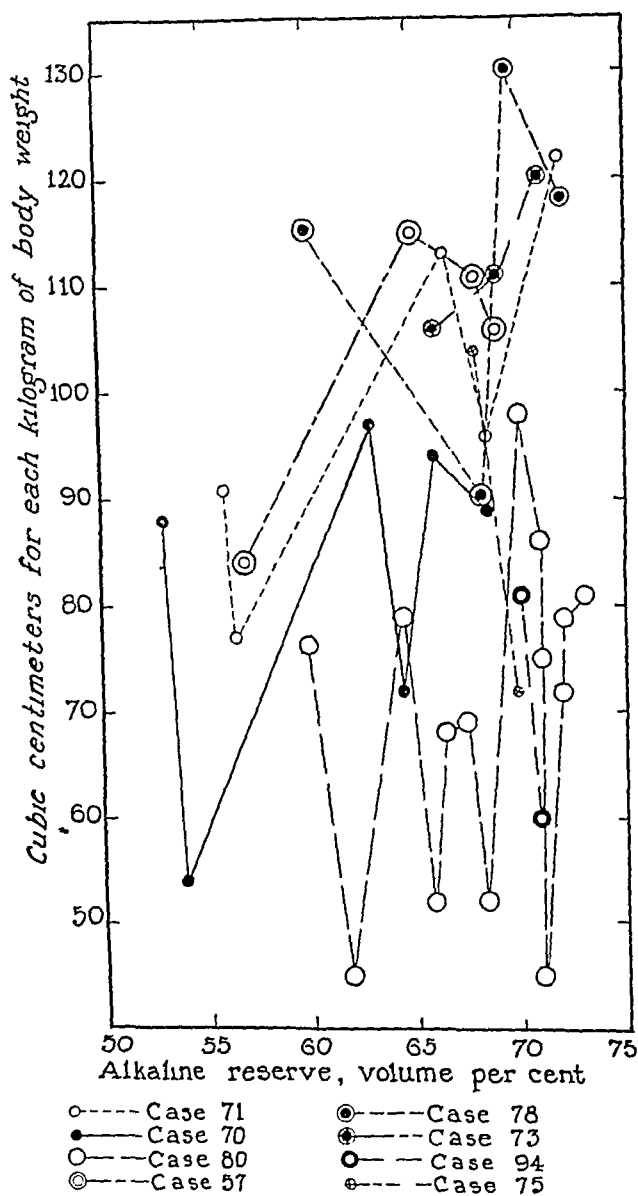


Fig 1—Alkaline reserve in eight cases of pernicious anemia, plotted against simultaneous blood volumes calculated in terms of cubic centimeters for each kilogram of body weight

As these results seemed to indicate a relationship between blood volume and alkalinity, an attempt was made to correlate the alkaline reserve with the apparent blood volume as indicated by the concentration of the unagglutinable corpuscles following a transfusion of unlike group

TABLE 2—*Correlation of the Total Red Cell Count, the Blood Volume and the Alkaline Reserve in Patients with Pernicious Anemia*

Case	Days	Red Cell Count in Millions	Corpuscles for Each Kilogram of Body Weight	C c of Blood for Each Kilogram of Body Weight	Alkaline Reserve, per Cent by Volume	Time
71	Transfusion, 500 c c					
	0	0 88	57×10^9	73		
	1	1 00			57	
	2	1 06			51	
	3	1 07	84×10^9	77		
	4	Transfusion, 500 c c				
	5	1 62	123×10^9	77	56 5	
	8	1 23	112×10^9	91	56	
	14	1 09	133×10^9	122	72	
	14	Transfusion, 500 c c				
	16	1 38	138×10^9	100		
	18			113	66 5	
	18	Transfusion, 500 c c				
	18	1 53	147×10^9	96	68 5	
70	Transfusion, 500 c c					
	0	1 02	55×10^9	54	54	
	1	1 28	96×10^9	72	64 5	Morning
	1				69	Evening
	2	1 17			57	
	4	0 88	77×10^9	88	53	
	4	Transfusion, 500 c c				
	5	1 11	108×10^9	97	63	
	8	1 40	131×10^9	94	66	
	11	Transfusion, 500 c c				
	14	1 79	159×10^9	89	68 5	
80	0	1 17			71 5	
	0	Transfusion, 500 c c				
	0	1 26	59×10^9	47	71	p m
	3			47	62	5 30 p m
	4			72	72	9 30 a m
	4	2 25	117×10^9	52	66	8 30 p m
	5	1 76	134×10^9	76	60	9 00 p m
	6	1 93	189×10^9	98	70 5	9 00 a m
	6	1 96	133×10^9	68	66 5	8 30 p m
	7	1 93	153×10^9	79	72	10 00 a m
	7	1 99	150×10^9	75	71 5	3 00 p m
	7	2 06	146×10^9	69	67 5	6 00 p m
	10	2 21	180×10^9	81	73	9 00 a m
	10	Transfusion				
	11	1 51	119×10^9	79	64 3	9 00 a m
	16	2 06	107×10^9	52	68 5	9 00 a m
	17			56	71	
57	0	1 15			57	
	0	Transfusion (second in series)				
	0	1 48	161×10^9	111	68	
	7	1 30	150×10^9	115	65	
	7	Transfusion				
	7				60	
	9	1 72	183×10^9	106	69	
	10	1 23	103×10^9	84	57	
78	0	0 71			70	
	0	Transfusion				
	0				67	
	1	0 96	114×10^9	119		
	4	1 12	101×10^9	90	68	
	19	1 54	200×10^9	130	69 5	10 00 a m
	19	1 42	163×10^9	115	60	9 00 p m
73	20	1 27	150×10^9	118	72	
	0	1 33			67	
	0	Transfusion				
	0	1 32	222×10^9	145		
	6	1 60	193×10^9	120	71	
	9	1 94	204×10^9	106	66	
	10	2 09			69 5	a m
					69 5	p m
					69 5	a m
	13	2 13	250×10^9	111		
	13	Transfusion			57	p m
	18				63	
	20					
	21	3 22				
	22				65	p m
	23	2 32			66	a m
	30	2 67			63	
	36	2 21			72 5	p m
	37	2 51			58	a m

TABLE 2—*Correlation of the Total Red Cell Count, the Blood Volume and the Alkaline Reserve in Patients with Pernicious Anemia—Continued*

Case	Days	Red Cell Count in Millions	Corpuscles for Each Kilogram of Body Weight	C c of Blood for Each Kilogram of Body Weight	Alkaline Reserve, per Cent by Volume	Time
94	0 Transfusion (third in series)					
	11	2 50	150×10^9	60	71	a m
	11				73	p m
	14	3 03	243×10^9	61	70	
75	0	1 90			66	
	0 Transfus on					
	0	2 22	160×10^9	72	70	
	3				65	
	3 Transfusion					
	3	2 48	258×10^9	104	68	
60		2 01			59	
					57	
Patients with Secondary Anemia						
76	0				62	
	0				63	
	16				62	
86	0	3 66	378×10^9	104	74 5	p m
	1	3 53	308×10^9	87	64 5	a m
	2	3 22	300×10^9	93	75 5	p m

blood The alkaline reserve was determined from the urine by the method of Van Slyke and Fitz³ (Table 2) The blood volumes, as determined by the count of unagglutinable corpuscles derived from the unlike group transfusion given at the beginning of each study, have not been included beyond the point at which it seemed improbable that there had been any elimination of transfused blood All the alkaline reserve determinations are included

Although the first two studies appeared to show a rather marked parallelism between alkaline reserve and blood volume percentage, this relationship did not hold with the study of further cases I therefore concluded that there was no relationship between the two, and that the apparent increases in volume following administration of carbonates in Cases 59 and 61 were a matter of accident On further analysis, however, there seems to be a slight tendency for alkaline reserve to increase when the apparent blood volume increases, but a tendency for parallelism becomes much greater when the blood volume for each kilogram of body weight is multiplied by the red cell count In Figures 1 and 2, alkaline reserve is plotted against the blood volume for each kilogram of body weight in one instance, and the number of corpuscles for each kilogram of body weight in the other The points that indicate the readings for individual patients are connected, but the general trend of the curves would seem to be more significant than the individual curves

³ Fitz, R, and Van Slyke, D D Studies of Acidosis, IV, The Relationship Between Alkaline Reserve and Acid Excretion, J Biol Chem **30** 389-400 (June) 1917

SUMMARY AND CONCLUSION

In patients with pernicious anemia, the alkaline reserve, as determined by the method of Van Slyke and Fitz, was frequently found to fall below the normal limits (from 63.1 to 77.5) given by those authors, but after transfusions it tended to be normal

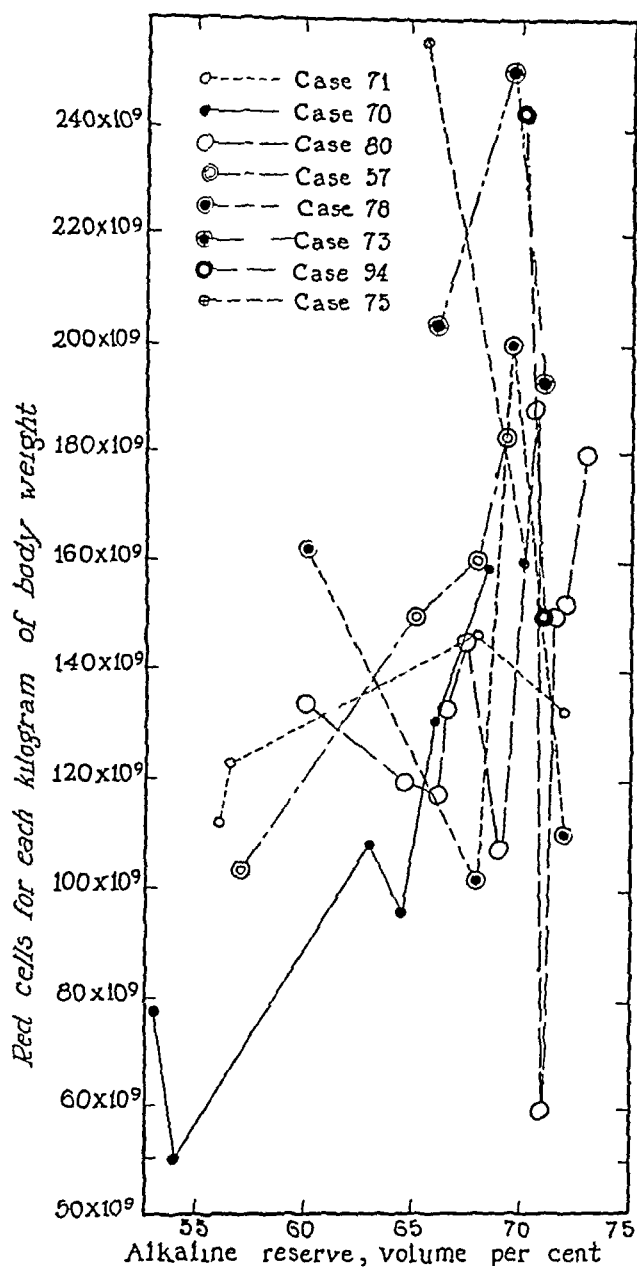


Fig 2—Alkaline reserve in eight cases of pernicious anemia, plotted against total number of corpuscles present for each kilogram of body weight

Although there is a great deal of variation, possibly due to variation in the amount of strain put on the carbon dioxide eliminating mechanism by exercise and so forth, it would appear from these data that there is a slight tendency for the alkaline reserve to increase with the blood volume, but that there is a greater tendency for the alkaline reserve to

increase with the increase in the total number of corpuscles present for each unit of body weight. In view of the fact that Henderson, Bock, Field and Stoddard⁴ consider that hemoglobin is responsible for the transfer of 90 per cent of the carbon dioxide from the tissues to the lung, this is not surprising.

The much more marked relationship between the hemoglobin content and the alkaline reserve than between the blood volume and the alkaline reserve would suggest that the increased alkaline reserve may merely be the effect of the increased carbon dioxide carrying capacity of the blood, and may not have any causal effect on increase in blood volume.

⁴ Henderson, L. J., Bock, A. V., Field, H. Jr., and Stoddard, J. L. Blood as a Physicochemical System, II, *J. Biol. Chem.* **59** 379-431 (March) 1924.

ACUTE INTESTINAL OBSTRUCTION

III SIMPLE OBSTRUCTION *

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AND

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During the last two decades, a vast amount of interesting work has been done on acute intestinal obstruction in an endeavor to find the lethal factors involved. Beyond keeping the subject alive in the experimental field, much of the work has added little to the ultimate settlement of the questions at issue. Complicated operative procedures, ether and morphin anesthesia, methods used because of preconceived premises of toxic substances, and the failure to recognize different types of obstruction have been the main stumbling blocks.

In the preceding papers,¹ we have demonstrated that experimental obstruction produced under local anesthesia gives most ideal results. The postoperative animals are in practically the same condition as the human patients seen clinically. The only complicating factors in the experimental work are the procain anesthesia and the 3 inch (7.6 cm) incision through the linea alba.

Carefully checked control animals on which only the abdominal incision has been made show that these two factors cause little or no systemic reaction.

Obstruction thus produced demonstrates clearly that there are two types of acute intestinal occlusion. (1) Acute strangulation. This includes those cases in which there is an interference with the venous, arterial and lymphatic circulation in the bowel wall and mesentery as well as complete obstruction of the intestinal lumen. Under this heading are grouped volvulus, strangulated hernia and intussusception. This condition is characterized by rapid pulse and respiration, subnormal temperature and low blood pressure, as well as the usual obstruction symptoms. Death here is due to shock, toxemia and peritonitis. (2) Acute simple obstruction. This comprises those cases in which there is a complete blockage of the bowel lumen only, with practically no circulatory involvements. Here the obstruction is produced by gall-

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1 Hausler, R. W., and Foster, W. C. Studies of Acute Intestinal Obstruction, I, Different Types of Obstruction Produced Under Local Anesthesia, *Arch. Int. Med.* **34**: 97 (July) 1924, II, Acute Strangulation, *ibid.* **34**: 697 (Nov.) 1924.

stones, enteroliths, foreign bodies, adhesions and bands The temperature, pulse, respiration and blood pressure show no marked deviation from the normal, even after ten days, although the obstruction symptoms are very pronounced

Acute strangulation has been dealt with in a previous paper In this paper, we shall endeavor to show that death in simple intestinal obstruction is not due to a toxemia, as is generally assumed, but instead is due to starvation

In experimental simple obstruction, occlusion of the bowel lumen is usually produced by clamps, ties or section of the intestine and inversion of the cut ends All three of these methods have their disadvantages and introduce certain complications which must be considered when interpreting the results obtained The ideal method of producing obstruction is naturally that seen in human cases, namely by bands of living tissue The aluminum clamps, as first used by Hartwell and Hogue², are not very satisfactory because of the difficulty in determining definitely whether the obstruction has been complete Tissue destruction, abscess formation and peritonitis are rather frequent complications Ligation of the intestine, with silk or cord ties, produces good results for several days, but unfortunately the ligature cuts through the intestinal wall in four or five days, and the bowel reunites or death follows from perforation and peritonitis Section of the intestine and inversion of the proximal and distal stumps gives a complete obstruction that is easily produced and very permanent This technic, however, causes considerable tissue destruction and is followed by a severe constitutional reaction The postoperative record shows that for the first five or six days, or until the inverted stumps have healed, there is a quite pronounced rise in temperature, pulse and respiration Unless subcutaneous injections of fluid are given during this reparative period, many of the animals die However, if the dogs survive, we have an ideal obstruction exactly comparable to that seen in human cases The obstruction is complete, and little or no devitalized tissue is present The writers have used all three methods and find that the section method gives the most satisfactory results

LITERATURE

No attempt will be made to review the extensive literature on the subject of intestinal obstruction Instead, the reader is referred to a recent article by Ellis

The literature shows five main theories as to the cause of death in acute intestinal obstruction

1 Bacteremia The theory that death is due to a bacteremia at first sight seems very plausible McClure³ has shown that the fluid accumu-

² Hartwell, J A, and Houget, J P An Experimental Study of High Intestinal Obstruction, *Am J Med Sc* **143** 357, 1912

³ McClure, R D An Experimental Study of Intestinal Obstruction, *J A M A* **49** 1003 (Sept 21) 1907

lating in the intestines above the obstruction acts as a very good culture medium. It furnishes all the requirements for bacterial growth, i. e., food, water, proper temperature and an alkaline reaction. Furthermore, the normal intestinal tract is the habitat of innumerable pathogenic bacteria. Under such ideal conditions, they multiply very rapidly and thus might easily work their way through the intestinal mucosa into the blood stream.

However, Krafft,⁴ McClure,³ and Hartwell and Hoguet² have definitely disproved this theory by showing that at death the heart's blood, peritoneum and organs are practically always sterile.

2. *Perverted secretion.* Supporters of this theory state that after obstruction, the intestinal mucosa of the proximal segment secretes a toxin which when absorbed is rapidly fatal.

Dragstedt, Moorhead and Burcky⁵ showed the fallacy of this theory by draining open isolated duodenal and jejunal loops into the peritoneal cavity. Their dogs lived indefinitely and showed no toxic symptoms.

3. *Shock theory.* The clinical evidence of tachycardia, low blood pressure and profound collapse, combined with wide dilatation of all splanchnic vessels, point to severe disturbance of the cardiovascular system.

These symptoms are true indications of shock and, as previously stated, appear in practically all cases of acute strangulation.

The protocols of dogs with simple intestinal obstruction, produced under local anesthesia, show an almost complete absence of shock symptoms. The pulse and respiratory rates are always practically normal, the temperature curve shows only the usual diurnal variation, and the blood pressure remains the same even after twenty days.

Shock, however, is an acute affair always manifesting its symptoms within the first twenty-four hours. These simple obstruction dogs live three or four weeks. It is thus evident that shock is not a lethal factor in simple intestinal obstruction.

4. *Toxemia.* This theory states that death in acute intestinal obstruction is due to the absorption of poisons produced by bacterial action on the stagnant bowel fluids which accumulate in the intestine above the obstruction. This theory, which has received almost universal

⁴ Krafft, quoted from Enderlain, Hotz. *Mitt. a. d. Grenzgeb. d. Med. u. Chir.* **23** 755, 1911.

⁵ Dragstedt, L. R., Moorhead, J. J., and Burcky, F. W. *Experimental Study of Intoxication in Closed Intestinal Loops*, *J. Exper. Med.* **25** 421 (March) 1917.

acceptance, has been supported by experiments demonstrating that death is quickly produced when these fluids are injected intravenously into normal animals. Thus far, no one has been able to demonstrate toxins in the blood stream or their absorption by the intestinal mucosa.

5 Dehydration. In 1912, McLean and Andries⁶ and Haitwell, Hoguet and Beekman,² each group working independently of the other, concluded that the water loss from drainage of the body fluids into the intestinal lumen above the obstruction was the cause of the systemic symptoms and the eventual cause of death.

This dehydration theory seems very plausible, since clinically we know that the mechanical obstruction causes an almost immediate vomiting of all foods and liquids taken, and, in addition, that the profuse vomitus contains a considerable quantity of bile, pancreatic, gastric and intestinal secretions.

The last two theories have never been definitely proved or disproved.

METHODS

The operative procedures used were substantially the same as those described in the preceding papers. All work was done on healthy dogs under aseptic technic. Blood chemistry estimations were made at frequent intervals by the methods previously reported. Blood pressure readings were made on the femoral artery under local anesthesia.

EXPERIMENTAL WORK

Rôle of Toxæmia—In our previous work on acute intestinal strangulation, it was noted that even massive doses of ten hour strangulated loop fluid was relatively nontoxic when injected intraperitoneally into normal dogs. This bloody exudate is an ideal culture media, even more so than intestinal secretions.

It thus seemed probable that the vomitus of simple obstruction was likewise nontoxic, especially since dogs of the simple obstruction group vomit at least three or four times a day, and thus prevent prolonged bacterial action on the accumulating fluid. Most high obstruction vomitus is not exposed to bacterial action for more than a twelve hour period. Selective action of the intestinal mucosa and vomiting also prevent much toxin absorption.

To determine, therefore, the relative toxicity of high obstruction vomitus, the total daily vomitus of four dogs with simple duodenal cord tie obstruction was collected, centrifugated, filtered through ordinary filter paper and slowly injected subcutaneously into normal dogs of approximately the same size. All vomitus was prepared, and injected as

⁶ McLean, A, and Andries, R. C. Ileus Considered Experimentally, J. A. M. A. **59** 1614 (Nov. 2) 1912.

soon as possible after it was obtained in order to prevent further bacterial action. The vomitus was not heated because of the objection that this might destroy the toxins as well as the bacteria. However, by this technic we were injecting millions of bacteria into the subcutaneous tissue of these dogs. Apparently, dogs have good resistance to bacteria since only one of these dogs developed skin abscesses following the three days' injection period.

Table 1 shows the most severe reaction noted.

Two days later, the dog had recovered entirely.

The experiments in Table 1 show practically no toxic effect from the absorption of this high obstruction vomitus, and we therefore conclude

TABLE 1—*Data Proving That High Duodenal Obstruction Fluid Is Relatively Nontoxic*

Dog 1, a male, weight 16 pounds (7.3 kg) Prepared vomitus injected subcutaneously

Day	Time	Vomit	Result
1	9 a m	60 cc	Vomit appeared to have no effect on dog
	12	60 cc	
	1 p m	60 cc	
	3	60 cc	
	5	60 cc	
2	9 a m	50 cc	Temperature, 103.2 Dog slightly indisposed
	12	65 cc	
	1 p m	60 cc	
	3	60 cc	
	5	60 cc	
3	9 a m	65 cc	Temperature, 103 Dog looked sick, evidently had bacteremia from injections
	12	60 cc	
	1 p m	50 cc	
	3	50 cc	
	5	50 cc	

that even the total daily vomitus is relatively nontoxic when injected subcutaneously, as soon as possible after it is obtained. Since we know that the fluid current is mainly out of the body by way of the upper intestinal tract and is very large, it is evident that only slight absorption of toxins can occur.

Death from toxemia thus seems very improbable. What then is the cause of death? Perverted secretion and bacteremia have been disproved. Shock, we have shown, is not present. Starvation and dehydration appear to be the only remaining possibilities.

Rôle of Dehydration—Most workers assert that since death is early and vomiting almost negligible, dehydration cannot be a prominent lethal factor. Their operations were invariably under ether anesthesia, and the animals usually died in from three to five days. No fluids were given subcutaneously, and little vomiting was noted.

On the other hand, in the experimental work of Hartwell and Hoguet,² Bacon and Anslow and Eppler,⁷ reports are made of the

⁷ Bacon, D. K., Anslow, R. E., and Eppler, H. H. Intestinal Obstruction, Arch. Surg. 3: 641 (Nov.) 1921.

marked daily fluid output and loss of body weight in dogs of the simple obstruction type. They, however, had given daily subcutaneous injections of large quantities of saline solution and, under these conditions, the vomitus and urinary excretions were naturally much larger than they would have been had they allowed the body fluids to be gradually depleted, as they are in the ordinary cases.

With this fluid loss in mind, the clinical literature was examined for reports of total fluid output in human cases of obstruction, but none were found.

TABLE 2—*Fluid Loss in Low Duodenal Obstruction*

Dog	Weight	Days	Urine and Vomit	Result
1	18.5 pounds (8.4 kg)	1	180 cc	Dead in 60 hours from perforation and peritonitis
		2	520 cc	
	15.4 pounds (7 kg)	3	100 cc	
2	43 pounds (19.5 kg)	1	610 cc	Recovered after 90 hours
		2	415 cc	
		3	180 cc	
	37.1 pounds (16.8 kg)	4	95 cc	
3	24 pounds (10.9 kg)	1	285 cc	Died after 68 hours from perforation
		2	335 cc	
	19.2 pounds (8.6 kg)	3	950 cc	
4	20 pounds (9 kg)	1	375 cc	Recovered on third day
	19.1 pounds (8.6 kg)	2	275 cc	
	17.2 pounds (7.8 kg)	3	195 cc	
5	11 pounds (5 kg)	1	100 cc	Recovered on third day
	10.1 pounds (4.5 kg)	2	115 cc	
	9.5 pounds (4.3 kg)	3	80 cc	
6	20.4 pounds (9.2 kg)	1	210 cc	Recovered on fifth day
	19.9 pounds (9 kg)	2	180 cc	
	19.3 pounds (8.7 kg)	3	240 cc	
	18.9 pounds (8.6 kg)	4	130 cc	
	18.1 pounds (8.2 kg)	5	65 cc	
7	30.5 pounds (13.8 kg)	1	250 cc	Recovered on fourth day
	29 pounds (13.2 kg)	2	185 cc	
	28.3 pounds (12.8 kg)	3	195 cc	
	27.8 pounds (12.6 kg)	4	105 cc	

We therefore experimented on a series of seven dogs with simple duodenal and ileal obstruction, an accurate record was kept of daily fluid loss by urine and vomitus. Operations were performed, under local anesthesia, to prevent the usual atonic postoperative conditions of the bowel. The dogs were kept in metabolism cages so constructed that all fluid excretions could easily be collected. The obstruction was produced by ligating the bowel with a heavy cord tied just tight enough completely to block the bowel lumen. These ties cut through the bowel wall at the end of four or five days, and the vomiting immediately ceased, thus indicating that the obstruction had been relieved.

These results, although observed over only a short period of time show that fluid loss is quite pronounced.

In another series of six dogs in which obstruction was produced by section of the intestine and stump inversion, this daily fluid loss was replaced by subcutaneous injections of physiologic sodium chlorid solution. The results were as follows. One dog died of snuffles on the fifth day, another was killed on the twelfth day because of a large skin abscess, and the remaining four lived fourteen, sixteen and seventeen days, respectively.

Table 3 shows the usual temperature, pulse and respiratory variations noted.

The foregoing results confirmed the work of Hartwell and Hoguet,⁸ who first showed that, in simple intestinal obstruction, if the fluid loss

TABLE 3—*Typical Temperature, Pulse and Respiration in Obstruction of Lower Jejunum*

Dog 2, weight 55 pounds (24.9 kg), a large, black male

Days Before	Rectal Temperature	Pulse	Respiration	Blood Pressure	Sodium Chlorid, Cc	Result
1	101.4	84	18	150		
1	99.2	86	20		250	
2	101.0	126	18		250	
3	102.2	102	22		300	
4	102	88	20		300	
5	102.8	84	18		300	Slight nasal discharge
6	102.4	88	20		300	
7	102	76	18		350	
8	101.9	90	10		250	
9	101.1	66	18		350	
10	100.4	58	16		460	Snuffles worse
11	100.4	56	16		350	
12	100.4	54	20		460	Snuffles better
13	101	58	22		350	
14	100.6	74	18		360	
15	101	92	20		340	
16	100.4	172	20	100 systolic	300	
17		Died				Weight 41 lbs (18.6 kg)

Neeropsy. Obstructed viscera grossly normal, no peritonitis, proximal and distal stumps well healed, lower 6 inches (15.2 cm) of proximal intestine dilated slightly, and walls hypertrophied.

is replaced, the animals will live two or three weeks. They also indicated that fluid loss is a very important lethal factor in simple intestinal obstruction. That dehydration, however, is the sole cause of death seems very improbable. It certainly does not explain the rapid death obtained in experimentally produced obstruction. Fluid loss through vomitus and excretions is never sufficiently pronounced in obstruction to cause death in one, five or even ten days. All high obstruction dogs died in less than a week unless given fluid subcutaneously. It seemed apparent, therefore, that in experimental work there must be some complicating factor due to the operative procedure.

A careful review of the literature showed that, of the three methods used in producing obstruction, only one gave satisfactory results. Cord

⁸ Hartwell, J. A., Hoguet, J. P., and Beekman, F. An Experimental Study of Intestinal Obstruction, *Arch. Int. Med.* **13**: 701-736, 1914.

tie and clamp occlusion were too uncertain and too temporary, and perforation and peritonitis were very common. With section of the intestine and inversion, the obstruction was very satisfactory, but the animals died in about five days unless given fluids subcutaneously. It appeared evident, therefore, that in the latter method the time during which healing of the inverted raw ends was taking place was a critical period. The animals that survived this stage should give ideal experimental results.

Consequently, on a series of four dogs, cut and inversion obstruction was produced, and physiologic sodium chlorid solution was given subcutaneously, during the first five days, to tide them over this reparative

TABLE 4—*Simple Obstruction of Lower Duodenum*

Dog 4, an adult, female collie, weight 34.2 pounds (11 kg.)

Days	Temperature	Pulse	Respiration	Vomitus, C c	Saline Solution, C c	Chlorids Mg per 100 C c	Carbon Dioxid	Nitrogen, Mg per 100 C c	Blood Pressure	Observation
0	102.2	110	18			298	43.2	23	not taken	
1	103.2	152	24		250					
2	102.9	144	24		250					
3	102.6	148	22		240					
4	103.1	145	24		240					
5	104	140	22		250					
6	103.6	135	22							Urine scanty during remainder of starvation period
7	103.5	140	20							
8	103	138	20							
9	103.1	135	20							
10	102.7	128	16							
11	103.4	128	18			320	46.2		138 systolic	
12	102.6	160	12							
13	102.6	164	18			298	60.49			
14	102.7	176	16							
15	102.6	176	12							
16	102.1	172	9							Mucosa of nasal and buccal cavity dry and crusted
17	142.1	172	11			310	53.6	40		
18	101.8	176	10							
19	102.7	160	10	150		320	35.8	54		
20	102.6	165	12						105	
21	102.1	Killed in afternoon				330	42.5	67		

Necropsy (done immediately) Weight 19.3 pounds (8.7 kg.) Abdominal viscera grossly normal, no peritonitis, stumps well healed, walnut sized abscess in mesentery just below proximal intestine. Weights of viscera, as follows: Liver, 272 gm.; kidneys, 76 gm.; spleen, 10.5 gm.; thyroid, 4 gm.; suprarenals, 3.2 gm.; pancreas, 21 gm. and ovaries, 1.4 gm.

period. Complete food and water starvation was then begun. One animal died on the third day from hemorrhage in the proximal segment, another developed snuffles and was sacrificed on the fifth day. The remaining two dogs, to our amazement, lived twenty-one and twenty-eight days, respectively. In other words, these animals lived sixteen and twenty-three days without food, water or salts with complete obstruction of the small intestine. Tables 4 and 5 show the post-operative results.

These two animals, especially during the last ten days, showed the typical picture of starvation. The loss of weight was extreme. The skin became dry and inelastic, and the shedding of hair was pronounced.

Folds produced in the skin flattened very slowly. Salivary secretion ceased entirely, and as a result the tongue and oral mucosa became crusted with foul smelling, dry serous plaques. The last five days, the nasal apertures became plugged with similar secretions and nasal breathing was harsh and difficult. The skin was extremely anesthetic so much so that incisions caused no pain. The extremities were cold and the blood flow through them of such small volume that the necessary amount of blood for chemistry estimations was difficult to obtain. The rectal

TABLE 5—*Simple Obstruction of Upper Jejunum*

Dog 5, an adult, female mongrel, weight, 51 pounds (23.1 kg)

Days	Temperature	Pulse	Respiration	Vomit, C c	Saline Solution, C c	Chloride, Mg per 100 C c	Carbon Dioxide	Nitrogen, Mg per 100 C c	Blood Pressure	Remarks
0	101.9	90	18			315	45.2	23	not taken	
1	103.2	148	18		350					Condition good
2	102.6	140	22		240					Condition good
3	102.1	126	20		240					Condition good
4	101.8	66	18		240					Condition good
5	102.9	64	18		250					Condition good
6	102.2	68	18		none					Condition good
7	102.3	64	18							Urine scanty
8	101.8	72	16							
9	102.2	68	18	300						
10	102	88	22							
11	103.3	96	20	130		310	60.4	40	143	
12	102.6	98	12							
13	102.4	126	20	250		300	66.5	38.2		
14	102.6	143	20							
15	101.5	160	18	15						
16	102.4	156	16	360						
17	102.1	138	16	180		300	56.4	46		Nose dry, respiration harsh, crusts on nose and lips
18	101.6	134	17							
19	101.6	114	16							
20	100.4	124	12							
21	100.1	146	14			270	53.7	45		
22	101	150	14							
23	101.2	140	12							
24	102.2	160	14	50		290	57	41.6		
25	101.8	132	15							
26	101.9	145	16						112	
27	101.8	160	16	410		280	62.7	42		
28	102.2	165	18							Sudden death

Necropsy (immediate). All thoracic and abdominal viscera grossly normal, no peritonitis, lower 3 inches (7.6 cm) of proximal intestine slightly dilated and walls hypertrophied. Distal segment very small and atrophic. Weights of organs, as follows: Liver, 254 gm; kidneys, 78 gm; spleen, 12 gm; thyroid, 3 gm; suprarenals, 3 gm; pancreas, 14 gm; and ovaries, 1.2 gm.

temperature was slightly subnormal, the pulse almost doubled in rate and the respirations were very slow. The blood pressure fell to about 110 mm of mercury systolic.

Throughout the experiment the dogs were active and playful and except for thirst, vomiting and the symptoms mentioned, appeared absolutely normal. At no time after the first five days was there any subjective or objective evidence of toxemia. The temperature, pulse and respiratory changes were certainly not those of toxemia, and the appearance and action of the animals were essentially those of a normal animal undergoing starvation.

The red blood cell count was consistently above normal after the first week, the counts varying between six and eight million. Hemoglobin estimations (Haskins-Osgood modification of Sahli) fluctuated between 120 and 135 per cent. Viscosity determinations were made by comparing the time required (stop-watch) for normal oxalated blood to flow from a special 5 c.c. viscosity pipet with that for the oxalated blood of the obstruction animals. This method, although not absolutely accurate, gave approximate results and revealed a quite decided increase in blood viscosity. This increase was also visibly apparent, the blood appearing thick and flowing very slowly. Clotting time was decreased, and no bleeding occurred following small skin incision. A comparison of the foregoing charted blood chemistry figures with those of Dog 5 of Morgulis and Edwards'⁹ starvation series (the only animal deprived of both food and water) shows that the changes are almost identical. The chlorids remain normal or slightly increase, the nonprotein nitrogen increases gradually although never to a very high figure, and the carbon dioxide combining power of the blood varies, usually slightly above normal limits. These changes are all indicative of the anhydremia of starvation.

A rather unique case of simple intestinal obstruction was seen by us several months ago and will be reported here, as it represents the type of obstruction produced by gallstones, foreign bodies and enteroliths. The owner of a large Airedale brought the animal to the medical school for treatment. He stated that the dog was poisoned about two weeks before, as a result of eating fresh salmon (common in Northwest). During this time, the dog had vomited all food and water eaten, had lost markedly in weight and strength, and when seen was in a precarious condition. Subcutaneous injections of physiologic sodium chlorid solution were given at once, but with little results, the dog dying on about the eighteenth day after the onset of the obstruction symptoms. At necropsy, a round basalt stone about 2 inches (5 cm.) in diameter, was found tightly lodged in the lower ileum above 5 inches (12.7 cm.) above the ileocecal valve. The small intestine above this point was markedly dilated, congested and contained a small quantity of fluid. No perforation or peritonitis was present, and the obstruction was complete. This case further substantiates the belief that starvation is the cause of death in simple obstruction.

COMMENT

It has been previously demonstrated that in uncomplicated cases of acute simple intestinal obstruction, bacteremia and perverted secretion

⁹ Morgulis, S., and Edwards, A. C. Chemical Changes in the Blood During Fasting and Subsequent Refeeding, *Am J Physiol* **68** 477 (May) 1924

of the intestinal mucosa are not the causes of death. Shock, the predominating lethal factor in acute strangulation of a large segment of intestine, is never present in simple obstruction. A review of the temperature, pulse and respiration and blood pressure curves of the accompanying tables shows a complete absence of shock symptoms. This theory, therefore, appears untenable.

That death is due to a toxemia is also very improbable for the following reasons:

- 1 The relatively slight toxicity of even large quantities of vomitus
- 2 The decreased absorption from the proximal segment
- 3 The fact that dogs with closed, isolated loops full of extremely toxic fluids live months without showing marked toxic manifestations
- 4 The quicker death in high obstruction (in this the function of the intestine is mainly secretory), and the slower death in low obstruction, in which normally much absorption occurs, and in which the intestinal secretions become more toxic because of delayed vomiting and more prolonged bacterial action

5 Furthermore, the entire clinical picture, namely, the temperature, pulse, respiratory and blood pressure variations and the general appearance of the patients and the experimental animals, certainly is not that of a toxemia.

6 In addition, the fact that, in uncomplicated cases of experimentally produced intestinal occlusion, the subjects live three or four weeks without food or water or any treatment whatsoever proves definitely that toxemia is not the cause of death.

The experimental workers supporting this theory have attempted to prove it by demonstrating that fluid from closed isolated loops is rapidly fatal when injected intravenously into normal animals. By chemical extraction, they conclude that the toxins are proteoses, nucleoproteins and histamin derivatives. Undoubtedly, intestinal secretions become very toxic when exposed to prolonged bacterial action. Death following intravenous administration, however, proves nothing, since it has long been known that many substances are extremely fatal when introduced directly into the blood stream, whereas if allowed to pass through normal intestinal mucosa their poisonous properties are entirely destroyed. Intravenous peptone injections may cause rapid death, yet the absorption of peptone from the normal intestine is, on the contrary, a natural physiologic process.

Furthermore, the absorption or presence of these toxins in the blood stream has never been demonstrated, nor has the introduction of blood from animals dying from obstruction ever produced untoward symptoms or death.

It is thus evident that, in uncomplicated acute simple obstruction, toxemia is not present, and that death is purely the result of starvation. All injected food and water is quickly vomited, and the result is therefore essentially the same as if these substances had been withheld. Fluid loss through urinary and respiratory excretion is probably similar in both starvation and obstruction. In addition, however, the obstructed animals are losing considerable quantities of the various juices secreted into the gastro-intestinal tract. In the normal starving animal these fluids are all reabsorbed. Gastric secretory loss, as Pawlow has indicated, is negligible after the fifth day of starvation. Pancreatic juice secretion probably shows a similar inhibition. Intestinal and biliary secretion, however, continues throughout the obstruction period, as indicated by the large quantity of bile stained vomitus. Experiments with biliary pancreatic and intestinal fistulas show that animals can survive these secretory losses for long periods of time, thus indicating that, in intestinal obstruction, this, in itself, does not cause death but instead merely hastens the starvation process.

That dehydration is very pronounced is evident. The dehydration theory, therefore, contains an element of truth. Fluid loss, however, is only a part of complete starvation for, in the latter, both food and fluids are withheld. Ordinary dogs deprived of all food and water live from fourteen to sixty-six days, with an average of about thirty days, whereas, if allowed water but no food, they may live more than four months. These figures indicate that death in simple obstruction falls within the same period of time as does ordinary starvation. The extra fluid loss in obstruction through vomitus merely accelerates the starvation process.

Furthermore, a review of the tables outlining the experiments on the two starvation dogs shows that changes are exactly similar to those reported for normal starvation animals. The increased viscosity of the blood, the high red cell and hemoglobin figures, the decreased blood volume flow through the extremities, the blood chemistry changes, the extreme loss of body weight and the temperature, pulse and respiratory curves have all been previously reported in starvation experiments.

The statement of Weielus that death in obstruction may be due to hepatic insufficiency and to a large amount of nonperistaltic bowel is not confirmed by our experimental work. Fluoroscopic examination of the proximal segments has always shown very active peristalsis even up to the time of death. That the distal intestine also is not aperistaltic, as generally stated, is proved by the fact that these obstruction dogs frequently pass small quantities of the white pasty feces that accumulate in the distal intestine. The vomitus is always darkly bile tinged and, during the last two weeks, appears to contain a gradually increasing amount of this hepatic secretion. At necropsy the fluid remaining in the proximal intestine is blackish green, and the gallbladder is distended.

with black, syrupy bile. Furthermore, blood sugar and urea estimations show no extreme variation from the normal, and the clotting time, instead of being increased, is, on the contrary, markedly decreased. Aperistalsis and ahepatism seem to play no rôle in acute obstruction death.

Experiments with obstructed mucosal extract have never warranted serious consideration.

Haden and Orr¹⁰ state that, in high, simple intestinal obstruction the characteristic changes in blood chemistry are a fall in chlorids, an increase in nonprotein nitrogen and, usually, an increase in the carbon dioxide combining power of the plasma. Since the nonprotein nitrogen increase does not begin until after the chlorids are well depleted, they conclude that the chlorids act as detoxicating agents. They also assume that the toxins are absorbed from the intestinal tract, and suggest that they cause much tissue destruction. An examination of their protocols shows that most of their dogs died within from two to five days after the obstruction was produced, namely, during the healing period unless treated by subcutaneous saline injection. Their conclusion, therefore, as to characteristic blood chemistry changes, toxin absorption and tissue destruction appears unwarranted. On the contrary, we find that the blood chemistry changes in untreated, uncomplicated, simple obstruction dogs are almost identical with those reported by Morgulis and Edwards⁹ for complete food and water starvation. The chlorids, instead of being decreased, remain normal or show a slight increase. The nonprotein nitrogen increases in the same ratio as that occurring in starvation. The carbon dioxide combining power of the plasma varies considerably, usually somewhat above the normal limits.

CONCLUSIONS

1 Death in uncomplicated cases of acute intestinal obstruction is due to starvation.

2 The blood chemistry changes noted in uncomplicated simple intestinal obstruction are almost identical with those found in complete starvation. Hypochloremia is not present.

3 Dehydration, in uncomplicated cases of simple intestinal obstruction, is usually very pronounced.

¹⁰ Haden, R. L., and Orr, T. G. Effect of Inorganic Salts on the Chemical Changes in the Blood of the Dog After Obstruction of the Duodenum, *J. Exper. Med.* **39** 321 (Feb.) 1924.

INTARVIN IN DIABETES *

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For the last three years, I have been feeding intarvin to diabetic patients and wish here to report the results thus far obtained. Most likely, this series of cases is the largest treated in this manner in one clinic.

Before I discuss the effects of intarvin, it is perhaps advisable briefly to review the rationale of this treatment.

Intarvin is the glyceryl ester of margaric acid. Its chemical formula is $(C_{16}H_{33}COO)_3 CH_2 CH CH_2$. We synthesized it in edible form in our laboratory. The theory underlying the therapeutic value of intarvin is, briefly, as follows.

In the wake of the intolerance for starchy foods that the diabetic patient evinces, there follows a disturbance in the assimilation of the fat foods, and this derangement is still more dangerous to the patient.

How do fats break down in the body?

It was shown by Knoop,¹ in 1905, that when fatty acids are given to animals in combination with aromatic radicals they are oxidized in a definite manner in the animal organism. It is known that if benzoic acid is administered by mouth it is excreted in the urine in conjunction with amino-acetic acid as hippuric acid.

If a higher acid than acetic acid is administered, the resultant product eliminated is either the hippuric acid or the phenaceturic acid, depending on whether the side chain was of an odd or even number of carbon atoms. Knoop, therefore, concluded that, in the oxidation of fats, the beta-carbon atom was oxidized and that two carbon atoms dropped from the chain together. This view was supported by Dakin's discovery that when phenyl propionic acid was given in large amount phenyl beta-oxypropionic acid was detected in the urine.

In his excellent summary, "Physiological Oxidations," Dakin² reviews the modern status of the theory of oxidation of fatty acids. I quote from him:

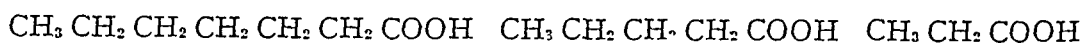
In spite of a good many vigorous onslaughts, the theory of beta-oxidation applied to fatty acids as put forward originally by Knoop has held its own. It will be recalled that one of the main supports of Knoop's views was furnished by Embden's observation that the normal fatty acids containing an even number of carbon atoms, varying from 4 to 12, all gave rise to aceto-acetic acid when their

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1 Knoop, F. Beitr. z. chem. Phys. u. Path. **6** 150, 1914.

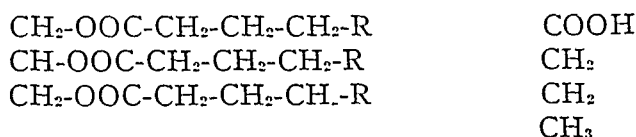
2 Dakin, H. D. Physiol. Rev. **1** 406 (July) 1921.

salts were perfused through a surviving liver. The normal fatty acids containing an uneven number of carbon atoms failed to cause any increase in aceto-acetic acid production. An interesting clue to the probable fate of some of these normal fatty acids has been furnished by Ringer, who finds that propionic acid is practically quantitatively converted into glucose in the phloridzinized dog and that normal valeric and heptylic acids given an amount of glucose under similar conditions comparable to the amount of propionic acid they might yield through beta-oxidation.



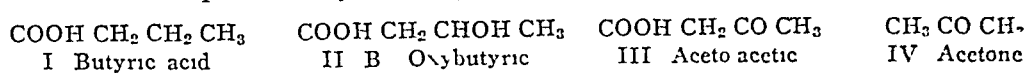
Blum and Woringer have recently shown that when propionic acid is given to normal dogs and rabbits, lactic and pyruvic acids are excreted, and these undoubtedly represent intermediary products of the oxidation of propionic acid, although it is not clear as to which of these two acids is first formed or whether perchance they are formed from acrylic acid, which Schwenken has shown to be almost quantitatively converted into glucose in the phloridzinized dog. The conversion of propionic acid into lactic acid explains the conversion of the former acid into glucose in the phloridzinized animal, for as is well known, the conversion of lactic acid into glucose under these conditions is virtually quantitative. The formation of lactic and pyruvic acids from propionic acid must be regarded as a case of alpha-oxidation, though possibly indirect, but since normal beta-oxidation with formation of ketonic acid is no longer possible with a three-carbon acid it need not be regarded as violating the beta-oxidation rule applicable to acids containing four or more carbon atoms. It appears, therefore, that in the metabolism of normal fatty acids containing four or more carbon atoms aceto-acetic acid is a common metabolite of all those with an even number of carbon atoms, while lactic acid is common to those with an uneven number.

In the catabolism of fats (under normal conditions, that is in the presence of proper carbohydrate oxidation) there is a rapid breakdown of the fatty acid radical to the four-carbon acid, i. e., butyric acid



The butyric acid is then rapidly catabolized to carbon dioxide and water.

This process, however, is markedly disturbed in states of deficient carbohydrate oxidation. In the latter circumstance, the fats are primarily broken down to butyric acid, as in the normal condition, but, in the absence of the heat of carbohydrate consumption, the further decomposition of the butyric acid proceeds very slowly. The butyric acid, under these conditions, is decomposed first to beta-oxybutyric acid and then to aceto-acetic (or diacetic) acid. The further decomposition to acetone takes place very largely in the urine itself.



Rosenfeld³ has said that fats burn only in the fires of carbohydrates, but this is not quite right, as only the breakdown of the lowest products of fat decomposition, that is to say, the acetone substances, depends on the catabolism of the carbohydrates.

This conversion to glucose does not take place in pancreatectomized dogs nor in diabetic patients, again demonstrating the great difference between phlorhizin glycosuria and true diabetes

Dr Sansum of the Potter Metabolic Clinic, Santa Barbara, Calif., draws the following conclusions from his experiments on intarvin. I shall conclude my paper by quoting these sentences

1 Intarvin, unlike natural fat, may be used in the diet of a patient with severe diabetes without fear of acidosis

2 The substitution of natural fat for intarvin in the amounts used in the experiment promptly brought on an alarming acidosis

3 The foregoing severe acidosis was promptly relieved when the intarvin was restored to the diet

Gies and Heft, in collaboration with me,⁶ report as follows

Eight normal, female rats from one litter were originally separated into groups of four. One of two males, of practically the same size and vigor from an unrelated litter, was added to each of these two groups. One group received a balanced natural diet with a definite proportion of intarvin mixed with the meat ration. The other group received the same natural diet under identical conditions, with rendered lamb fat instead of intarvin added to it. In each successive generation, thereafter, the maternal rats that received intarvin were selected from the direct descendants of those that had previously been given the "intarvinated" diet. The control maternal rats in each generation in a series were selected from the direct descendants of those that received a diet containing an addition of lamb fat. The paternal rats in each generation (unrelated to the females) were selected on the same dietary basis as that for the maternal, or from litters on the ordinary diet without addition of fatty matter.

Rats have been carried, in this parallel way, into the fourth generation in one series and into the third generation in another. (The experiments are in progress, and litters in the fifth and fourth generations, respectively, will soon be available.) There has been no discernible effect on the animals thus treated, or on their fecundity. The animals in the third and fourth generations appear to be as completely normal as those with which the tests were started last August.

The tissues of many of the rats have been used in the studies of the absorption and distribution of glyceryl trimargarate.

Hubbard and Wright have obtained the following results. I shall quote several of their sentences.

Haldane has shown that if large doses of sodium bicarbonate (0.6 gm per kilogram of body weight) are fed to normal subjects receiving normal diets the metabolism of glucose is depressed and acetone bodies appear in the urine. An experiment was planned to determine whether an increased excretion of acetone could be induced by moderate doses of the alkali in an arthritic subject who was receiving a diet which furnished sufficient calories for her needs (the patient gained in weight during the period of study) but which contained such small amounts of carbohydrate as just to prevent any significant ketosis. A markedly increased excretion of acetone was induced by 13 gm (20 grains) of sodium bicarbonate fed in three equal doses after each meal. The increased excretion lasted as long as the drug was continued (from August 11 to 18). The diet was approximately constant throughout the experiment except on the 17th and 18th of August, when the carbohydrate was somewhat decreased.

through an error. After this experiment part of the fat in the diet was replaced by a synthetic fat containing fatty acids with an odd number of carbon atoms (intarvin). A reduction in the amount of acetone excreted was found on the 22d and the 23d, as would be expected from the work of Kahn. The carbohydrate content of the diet was then slightly decreased, and again intarvin was substituted for part of the fat (August 28) and an even greater decrease in the excretion of acetone than before was found. In this experiment on a subject maintained at the borderline of ketosis, therapeutic doses of sodium bicarbonate caused an increased, and the synthetic fat intarvin a decreased excretion of acetone.

Lundin⁷ and Modern⁸ confirmed my results as to the non-production of acetone substances from intarvin. The results they have otherwise obtained are highly interesting, if not illuminating, and my critique of their work will appear in the *Journal of Metabolic Research*.

In feeding intarvin, the following plan is followed. The diet of the patient is determined according to the conventional method, i. e., it is determined how many grams of fats, carbohydrates and protein the patient can take in without the formation of acetone or the excretion of glucose in the urine. If the patient needs insulin, it is administered to him according to the orthodox system, after the proper diet for such a subject has been computed.

Now, having administered this proper diet of *natural* fats, carbohydrates and proteins, the caloric value yielded is calculated, and to this value is added a sufficient weight of *artificial* fat (intarvin) to bring up the caloric intake to a good maintenance diet. Thus, for example, if a patient without insulin can only take in 800 calories a day, and with insulin 1,800 calories a day, intarvin may be added to bring up the caloric intake to say 2,500 calories a day. If a patient, for any reason, does not receive insulin (whether insulin has no effect, or the patient does not require insulin, or refuses to take it), a quantity of intarvin may be added sufficient to make the tolerance diet a maintenance diet.

Intarvin may be administered in any form. It has been reported to me that exposure of intarvin in a plate to the direct rays of the sun takes away its flavor (slight as it is). I have found that it can easily be taken dissolved in hot black coffee or tea. If the physician does not impress the patient with a psychologic distaste, the patient will not observe any disagreeable flavor or odor. It surely is more tasteful than any emulsion of cod liver oil.

SUMMARY

The results obtained in patients may be summarized as follows

- 1 It nourishes them, so that they gain weight
- 2 It allays their sense of hunger
- 3 It strengthens them, so that they do not feel so "weak and tired," the usual complaint of the diabetic patient

4 It never produces ketosis, i. e., beta-oxybutyric acid, diacetic acid or acetone

5 It never produced acidosis. This I have determined in more than 100 cases by examination of the blood for the carbon dioxide combining power

6 It never yields glucose

7 Many more patients can be treated with intarvin, and thus insulin can be preserved for the patients with very severe diabetes who really need it

METHODS OF RECOGNIZING SCAPULAR TYPES IN THE LIVING¹

WILLIAM WASHINGTON GRAVES, M D

ST LOUIS

My investigations¹ led to the classification of scapulae (convex, straight and concave), and brought the scapula into definite relation with a number of biologic problems. Among these may be mentioned *morphogenesis and ontogenesis in general*, bone growth, bone form and variation in man and other vertebrates, the modification of bones by muscle attachments, muscle pull and various environmental influences in prenatal and postnatal periods, age changes in bone and other tissues, human heredity, human longevity, human morbidity, and human adaptability in general. The relations of the scapula to the foregoing problems and the desirability of establishing uniform and dependable methods of recognizing scapular types in the living are shown by recalling some recent findings.

SUMMARY OF SOME RECENT FINDINGS

1. Some recent findings are. Similar scapular types are present in varying degrees and percentages in the skeletal remains of all accessible human races and stocks both ancient and modern.

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1. Graves, W. W. The Scaphoid Scapula, A Frequent Anomaly in Development of Hereditary, Clinical and Anatomical Significance, *Med. Rec.* **78** 861-873, 1910, The Clinical Recognition of the Scaphoid Type of Scapula and Some of Its Correlations, *J. A. M. A.* **55** 12-17 (July 2) 1910, Some Remarks on the Scaphoid Scapula and Its Syndrome, *Tr. Nat. A. Study of Epilepsy* **8** 56 (June 16) 1911, The Scaphoid Scapula Syndrome, Its Connection with Syphilis in the Ascendants, *Interstate M. J.* **18** 109-116, 1911, also translated in *Deutsche Ztschr. f. Nervenheilk.* **41** 247-257, 1911, Scapula scapoidaea, eine häufig vorkommende Anomalie des Schulterblattes, ihr Zusammenhang mit Syphilis in der Aszendenz, *Med. Klin.* **7** 208-301, 1911, Einige Bemerkungen über die Scaphoid-skapula und ihre Begleiterscheinungen, *Wien. klin. Wchnschr.* **25** 245-238, 1912, Remarks on the Scaphoid and Its Syndrome, the Connection with Syphilis in the Ascendants, *J. Cutan. Dis. incl. Syph.* **31** 241-245, 1913, The Age Incidence of the Scaphoid Type of Scapula. Its Bearing upon Problems of Racial Morbidity, *Contributions to Medical and Biologic Research*, New York, Paul B. Hoeber, **1** 525-532, 1919, An Appeal for Embryos and Fetuses, *J. A. M. A.* **73** 1788 (Dec. 6) 1919, Discussion. The Scaphoid Type of Scapula, *Am. J. Syph.* **4** 478, 1920, The Types of Scapulae. A Comparative Study of Some Correlated Characters in Human Scapulae, *Am. J. Phys. Anthropol.* **4** 3, 1921, Observation on Age Changes in the Scapula, a Preliminary Note, *Am. J. Phys. Anthropol.* **5** 21, 1922, The Age Incidence of Scapular Types. Its Possible Relation to Longevity, *Tr. Am. A. Life Insurance Medical Directors*, 1923, The Relations of Scapular Types to Problems of Human Heredity, Longevity, Morbidity and Adaptability in General, *Arch. Int. Med.* **34** 1-26 (July) 1924, German translation in *Ztschr. f. Konstitutionslehre*, to be published.

2 Scapular types are inherited morphologic features, and with the exception of definite racial marks of inheritance, in no accessible morphologic feature common to "normal" human inheritance thus far known do the members of a given generation more closely and more frequently resemble each other and at the same time one or both parents than they do in scapular types

3 In the tenth to twelfth fetal week the human scapula has attained the general form it apparently ever afterward retains, and in this early period of development the same scapular types are found as in all subsequent periods of prenatal and postnatal existence

4 In prenatal and postnatal periods one finds scapular type asymmetries, "mixed types," in the same person. These may be convex on one side and on the other concave, or convex on the one side and straight on the other, or straight on one side and concave on the other. Such asymmetries in type are independent of right or left-handedness, occupation, disease or other environmental influences. Moreover, similar mixed types are found in some other mammals

5 In all postnatal periods of life, each scapular type (convex, straight and concave) is found in varying degrees and percentages, regardless of race, stock, sex, social level, occupation and environment. They are found in the tall, the short, the fat, the lean, the "broad backs," the "narrow backs," the weak, the strong, the healthy, the sick, the near to, and the remote from, an ideal in development, the mentally brilliant, the average and the defective, the well and the poorly muscled, the "excellent," the "good" and the "poor" types, and in all heretofore described human types

6 It is among persons disclosing innate defectiveness in adaptation, those disclosing definite asymmetries, disproportions and disharmonies in total make-up, that the straight and concave types are most frequently found

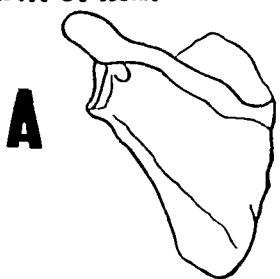
7 Investigations of various groups of human beings representing living, necropsy and skeletal material of known age disclose a finding as yet unparalleled in mammalian morphogenesis, *the age incidence of scapular types*, that is, it has been found that the convex increases while the straight and concave decrease in frequency of occurrence during successive age periods from birth to old age

8 It also has been found that no process or circumstance as yet known changes one scapular type into another after the earliest type formation, hence the inference is tenable that the straight and concave are more often found than are the convex in the plus potentially sick the shorter lived of the race

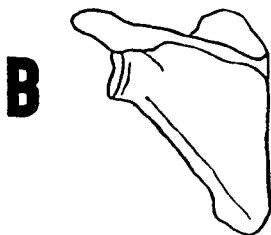
From the foregoing summary it becomes apparent that the human scapula has definite relations to the problems mentioned and particularly

to those of human heredity, morbidity, longevity and adaptability in general. These are vital, practical and enduring problems, requiring universal and continued research. They are enduring because they are founded in differences and similarities (variations) apparent in the individuals of various species, and in none more so than in the human

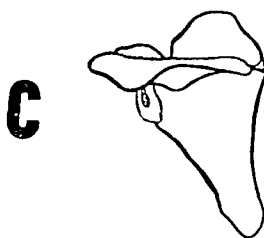
GRAVES'
SCAPULAR CLASSIFICATION
CONVEX-STRAIGHT-CONCAVE
 BASED PRIMARILY ON CHARACTER OF GREATER PORTION OF VERTEBRAL-BORDER CONTOUR BELOW SCAPULAR SPINE.



THE **CONVEX** TYPE: MAY BE REGULARLY OR IRREGULARLY, SLIGHTLY: C_v1, MODERATELY: C_v2, OR MARKEDLY: C_v3 CONVEX.



THE **STRAIGHT** TYPE: STRAIGHT OR NEARLY SO, NEITHER CONVEX NOR CONCAVE, TENDING RATHER TO CONCAVITY THAN TO CONVEXITY.



THE **CONCAVE** TYPE: MAY BE REGULARLY OR IRREGULARLY, SLIGHTLY: C_c1, MODERATELY: C_c2, OR MARKEDLY: C_c3 CONCAVE.

THE **SCAPHOID** TYPE COMBINES 12 OR MORE ANATOMICAL AND ARCHITECTURAL FEATURES COMMON TO STRAIGHT AND CONCAVE WHICH CONTRAST VIVIDLY WITH SIMILAR FEATURES IN THE CONVEX.

GRAVES AM J PHYS ANTHRO
VOL IV NO 2 1921

Fig 1—Scapular types, drawings by P. A. Conrath. A, convex type (C_v2), B, straight scaphoid type (St), and C, concave scaphoid type (C_c3).

The helpfulness of scapular classification in the prosecution of these problems will depend on the ease and reliability with which scapular types can be recognized in skeletal and living material. It is therefore the prime purpose of this article to point out dependable methods applicable to the living. This seems all the more necessary since the

recognition of scapular types represents a phase of the physical examination unknown prior to the classification of scapulae in 1910

BASES OF SCAPULAR CLASSIFICATION

Probably no bone in the body discloses as many morphologic and architectural characters as the scapula. Each of these characters shows a range in variation, but none a wider range than the vertebral border below the scapular spine. The variation-range of this part of the bone is from extremely convex to extremely concave or vice versa. Consideration of this fact enables one to classify the scapulae of various human races and stocks and of some other mammals into convex, straight and concave types. The classification of scapulae in human skeletal and living material is based primarily on the character of the greater portion of the vertebral border contour below the scapular spine in its relation to a straight line. By "greater portion" is meant the contour of the vertebral border between its point of mergence with the scapular spine and a point where it merges with the inferior angle region. The contour of the "greater portion" of this part of the vertebral border may be regularly, irregularly, slightly, moderately or markedly convex or concave, or it may be straight or nearly so, tending rather to concavity than to convexity. The classification is based secondarily on an assemblage of variations peculiar to, or most frequently found in, each type. Thus one type of scapula is brought into more or less sharp contrast with the other types not only by the character of the vertebral border contour (convex, straight or concave) but also by a number of correlated characters. While each scapular type embodies a variable constellation of correlated characters, it is nevertheless the relation of the contour of the greater portion of the vertebral border below the scapular spine to a straight line that establishes a firm foundation for scapular classification in skeletal and living human material. Figure 1 summarizes and pictures the primary basis of scapular classification and suggests useful symbols in recording the types and varying degrees of convexity and concavity.

METHODS APPLICABLE TO THE LIVING

It is a principle in clinical medicine and anthropometry to measure everything that can be measured with instruments of precision, but some of our most important findings are secured by comparing things and by exercising our powers of observation. It is with such methods that scapular classification must in the main be made. In approximately 6,000 skeletal scapulae, I have found only twenty-two mature bones, less than 0.5 per cent, in which classification was doubtful or impossible. As it depends on the factors of age, mental state, the nature of the

material and the personal equation, classification is obviously more frequently doubtful or impossible in the living. The methods applicable to the living are founded on the proper use of inspection, palpation and roentgen ray. In any part of the physical examination, the subject must be capable in some measure of cooperating with the examiner, hence the age or mental state of the person may wholly prevent classification or render it uncertain. In subjects under 3 years and especially in infants, in the definitely feeble-minded in all ages, in epileptics and demented, and in negativistic, resistive and excited insane, scapular classification is found to be difficult or doubtful in many and impossible in some. In the very obese in any period of life, classification can never be accurate, except with the aid of the roentgen ray. Accuracy even with this method depends on the use of proper precautions in minimizing or in preventing shadow distortion. To these ends, if the fluoroscopic examination is made, the subject should stand squarely before the target while the fluoroscope is placed squarely over the scapular region. To secure a reliable picture, the subject should rest with the scapular region squarely on the plate with the arms close to the sides and the forearms in positions of moderate supination. The exposure should be made while the subject holds his breath at the end of a deep inspiration. Prior to the sixteenth year of life, the roentgen ray is not dependable because the vertebral border and the inferior angle regions are still mainly cartilaginous, and the vertebral border cartilage is practically transparent to the ray in the living.

Depending on the age, mental state and nature of the material encountered the difficulties in scapular classification are very real, but they are practically negligible with proper methods under normal conditions. There is, however, another difficulty common to every phase of the physical examination, whether made with instruments of precision or other means, and this is the personal equation. The use of proper methods reduces this difficulty to a minimum, but it can never be wholly eliminated as long as the physical examination must be made with the imperfect mechanisms common to human beings. It is only by being conscious at all times of one's own imperfections and of the sources of error that one may secure results in scapular classification comparable in accuracy to other phases of the physical examination. It is in carefully carrying out the methods of inspection and palpation in orderly sequence, remembering the sources of error and in utilizing certain bodily correlations of scapular types that the personal equation with these methods can be reduced to a minimum and a high degree of accuracy can be secured. Let us now direct our attention to some of the bodily correlations of scapular types and to avoidable pitfalls in classifying them when inspection and palpation are employed.

Inspection—Classification by whatever method should never be attempted through the clothing. While the subject is stripped at least to the waist line and standing at ease with the shoulder girdle muscles relaxed and in good illumination, one should note the general development, the bodily habitus and attitudes, the topography of the trunk and abdomen, the form of the chest, the course of the clavicles, the right and left acromial level (height), the length of the neck, the spinal curves, the general muscular development, the dimensions and form of the interscapular space and the relation of the vertebral borders to the vertebral axis. In noting these points, one cannot avoid seeing others, such as nearness to, and remoteness from, good degrees of symmetry, proportion and harmony of the entire physical make-up, and variations about the skull, face and other parts of the body. All of these are helpful in the recognition of scapular types. During inspection of a number of persons, one quickly becomes aware of variations more frequently associated with one scapular type than with others, not only in the parts but in the total make-up of the subject. Among the variations that appear to be more frequently correlated with straight, and especially concave, than with convex types are rather sluggish bodily attitudes, "narrow back" habitus, meager or moderate musculature, long neck, drooped, hanging or rounded shoulders, chicken and funnel-shaped chest, long and flat chest often unduly flattened about the upper third anteriorly either unilaterally or bilaterally, narrowness of the costosternal angle, a horizontal or transverse course of one or both clavicles, prominent sternoclavicular and acromioclavicular joints, postural spinal curves of varying nature and degree, a difference in acromial level, and the relatively frequent finding of a flattened or retracted appearance of the upper third of the chest anteriorly on the low shoulder side. Moreover, one seldom finds in any age period Cc2 in "broad backs," but Cc2 and Cc3 types are relatively frequent findings in "narrow backs" prior to the fiftieth year of life.

The modeling of the vertebral border below the scapular spine is readily seen, except in the obese and the unusually well muscled. One frequently notes that the vertebral borders and the inferior angles are prominent (winglike), and these findings may be present in any type, but "winging" is more frequently associated with straight and concave types than with the convex (Figs 2 and 3).

Differences in dimension and form of the interscapular space are, in themselves, often dependable signs of type. The interscapular space is usually narrowed in the convex and widened in the straight and in the concave because the average of each of three scapular indices is less in the straight and in the concave than in the convex (Figs 2 and 3). Usually in the convex the form of the space is roughly A shaped, the general

direction of the vertebral border is downward and lateralward, and the inferior angle of each bone is then from 1 to 4 cm further away from the vertebral axis than is the base of the scapular spine (Fig 2*A*, Fig 3*B*) In the straight or concave, the base of the scapular spine

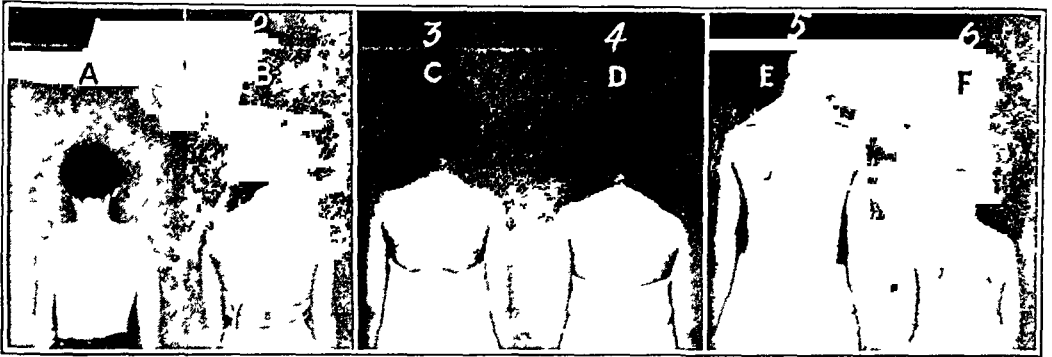


Fig 2—*C* and *D* are sisters, *E* and *F*, brother and sister, all subjects are standing at ease, shoulder girdle muscles relaxed, showing modeling of each vertebral border contour below the spine and the dimension and form of each interscapular space. Narrowness of space in *A* should be noted, broadness in *B*, *C*, *D*, *E* and *F*. The space is A-shaped in *A*, H-shaped in *B* and *F*, V-shaped in *C*, *D* and *E*. *D* has the Cc2 type on the left and the Cc3 on the right, and the right shoulder is definitely lower than the left. The other subjects have symmetrical types, and the shoulders of each are approximately equal in height.



Fig 3—Subjects standing at ease, shoulder girdle muscles relaxed showing modeling of vertebral border contour below spine. Broadness of interscapular space should be noted in *A* and narrowness in *B* and *C*. The space is A-shaped in *B*, H-shaped in *A* and *C*. *C* has Cv2 type on the left and Cv1 on the right, and the right shoulder is lower than the left. *A* has the straight type on the right and the left, *B* has Cv2 type on the right and the left, and the shoulders of each are approximately equal in height.

and the inferior angle are usually equidistant, or nearly so, from the vertebral axis and the form of the interscapular space is H shaped. The borders are parallel, or nearly so, to the vertebral axis (Fig 2*B* and *F*, Fig 3*A*). In some instances, however, in any type but more fre-

quently in the straight and concave, it is roughly V shaped. The general direction of each vertebral border is then downward and medialward (Fig 2C, D and E)

Another point as an aid to accuracy in scapular classification is the difference frequently noted in the acromial level (height), left and right. This difference has long been recognized especially by non-professional observers, and it is commonly believed to be due to previous or present disease or injury or occupation. The most frequent cause, however, appears to be differences in degree of what I² have elsewhere called the long scapular-axis-spinal angle, i. e., the outer inferior angle formed by the long scapular axis and the scapular spine. This angle is

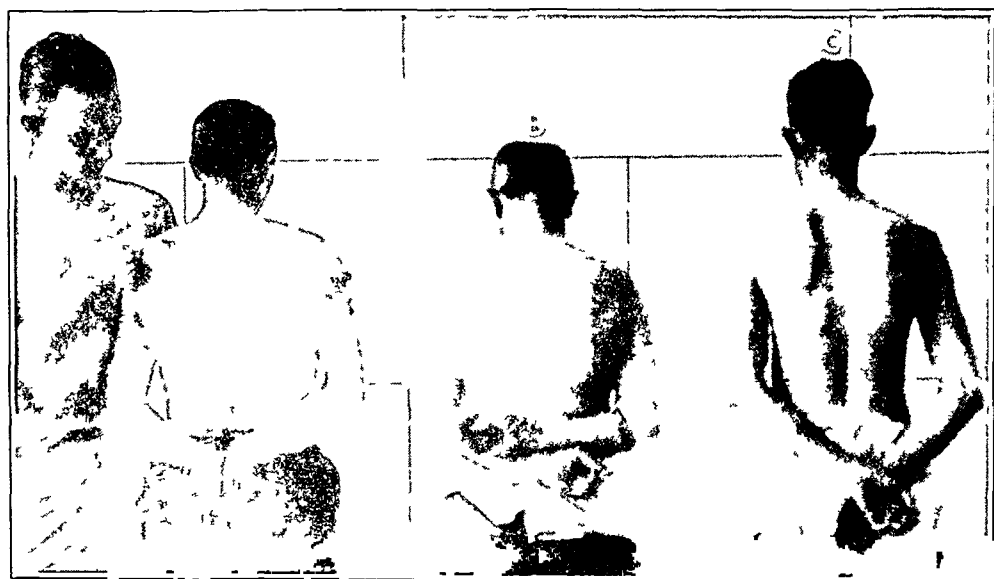


Fig 4—Same subjects as shown in Figure 3 in three useful upper extremity postures to relax rhomboid, trapezius and serratus muscles. Such postures render the vertebral borders more prominent for inspection and palpation. The person at the left is locating the base of the scapular spine.

usually definitely obtuse in the convex and more nearly a right angle in the straight and concave types. Thus, if a living subject possesses a convex on the right and a straight or concave on the left, the acromial level is usually lower on the left than on the right. The correlation of the varying degrees of this angle is not only present in subjects possessing one type of scapula on one side and another type on the other, but it is also present in subjects having differences in degree of a type. For instance, if the left scapula is Cv3 and the right Cv1 or 2, the right is usually lower than the left (Fig 3C). Similarly, if the right scapula is Cc3 and the left Cc1 or 2, the left shoulder is usually lower. Marked differences in acromial level are most frequently found in subjects possessing on one side a convex and on the other a

² Graves (Footnote 1, eleventh reference)

concave But these differences are seldom missing in persons who have straight on one side and concave on the other and in those having different degrees of concave types (Fig 2D), or of convex types (Fig 3C) The differentiation of one type from the others and the determination of the varying degrees of convexness and concaveness can come only from combining inspection and palpation and in the obese from using the roentgen ray

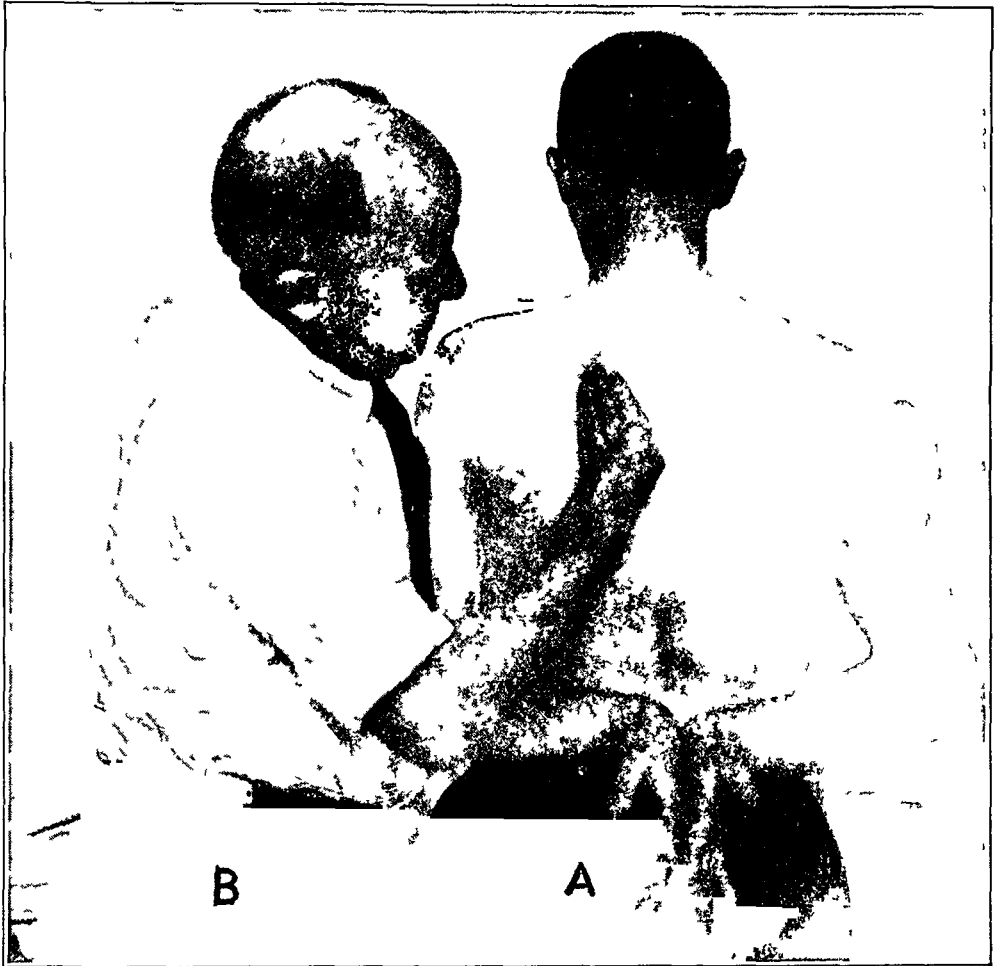


Fig 5—*B* and *A* shown in Figures 3 and 4, *B* is bringing lateral pressure to bear on the shoulder girdle of *A*, rendering the vertebral border still more prominent for inspection and palpation and testing relaxation by placing the side of the hand somewhat beneath the vertebral border

Palpation—The subject should stand at ease in natural attitudes without tensing any of his muscles, and particularly those of the shoulder girdle During palpation, the examiner should neither see nor feel contractions of these muscles and especially not of the rhomboid and the trapezius If contractions are seen or felt during palpation, the findings may be faulty, for example, a straight or a moderately concave may be called a convex These muscles are usually in a good state of relaxation when the patient stands at ease, but they can be further relaxed by certain upper extremity postures, for instance, by crossing

the subject's pronated hands over the sacrum or by placing the forearms over the small of the back, or by approximating the backs of the wrists over the small of the back (Fig 4) Posturing the upper extremities in any of these positions renders the border contours more prominent for inspection and palpation In such postures the serratus anterior muscles become relaxed, and the relaxation of these muscles is a further aid in rendering the vertebral borders prominent

To secure greater muscular relaxation, thereby rendering the vertebral borders still more prominent, i e, further elevating them from the chest wall, the following method is recommended If the examiner is right-handed, he should face and stand close to the left side of the subject, place his left hand over the upper portion of subject's right arm and bring rather firm pressure to bear on the subject's shoulder girdle regions between his own left arm and chest (Fig 5) With complete relaxation secured, one can now readily place the side of the hand or finger tips somewhat beneath each vertebral border (Fig 5) If one can do this, he can then be sure that he has secured the desired relaxation of the rhomboid, trapezius and serratus muscles Having located the base of the scapular spine and the inferior angle, palpation should now be made of the vertebral border contour throughout its extent below the spine The palpating fingers should be held at a right angle, or nearly so, to the border Palpation should be made slowly and firmly and without pain or discomfort to the subject Undue haste or force defeats proper palpation by exciting muscle contraction and resistance One should not only palpate under control of vision for convexness, straightness or concaveness of the "greater portion," but also for the minutest variations along the border contour

It is helpful to study border contour variations in good photographs of bones or, better still, in bones Some of these variations may be confusing to the beginner, and should here be mentioned Vertebral border contours uniformly or regularly smooth, uniformly or regularly convex, straight or concave are not usual findings The contour is more often somewhat uneven or irregular in convexness, straightness or concaveness In any type but especially in the straight and slightly concave, the border contour is often broken by one or more "buds" or "processes," varying in size and location A frequent finding in the straight and an occasional finding in the concave is a vertebral border prominence variable in size just above the inferior angle A relatively frequent finding especially in convex types, is a concavity of variable depth and length at the base of the scapular spine or just above the inferior angle In either case, the greater portion is usually convex in some degree In any border contour presenting a concavity in both locations, the intervening part representing the "greater portion" is also usually convex in

some degree. The greatest degree of convexness or concaveness may be found in the upper, middle or lower third of the border. The foregoing relatively common minor variations should lead to but little confusion if one constantly remembers the primary basis of scapular classification—the character of the greater portion of the vertebral border contour below the scapular spine in its relation to a straight line. The classification of each scapula should be determined, also the degree if found to be convex or concave, and the findings recorded. A record should also be kept of those cases in which scapular classification is found to be impossible and the reason for nonclassification should be stated whether obese, resistant or anatomic. (For methods of recording and tabulating scapular types the last reference in Footnote 1 should be studied.)

COMMENT

I have here given methods of recognizing scapular types in the living which I have found to be dependable, and have pointed out a few helpful bodily correlations and some pitfalls of error. It is believed that these methods will enable others who have proper training in clinical medicine to develop an accurate technic and to reduce the personal equation to a minimum. These are prime purposes in the application of every method, medical or otherwise. The work of others in similar material and in similar age periods in scapular classification has yielded very similar percentages to those secured by me. These results indicate that the difficulties in scapular classification in suitable material plus the personal equation should not yield a total error greater than 5 per cent. The results thus far secured have demonstrated the dependability of the methods here given. Scapular types are common to ancient and modern man, and they are inherited variations of unusual constancy, hence they are dependable bases for correlations in routine clinical examinations and in comparative studies of individuals, of families and of various stocks and races. The definite relation of scapular types to the problems of human heredity, longevity, morbidity and adaptability justify the conviction that continued application of scapular classification to these vital and enduring problems will eventually yield rich rewards to the physician and to the race.

THE VALUE OF CLINICAL CARDIODYNAMIC RECORDS *

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Methods of functional diagnosis should comply with two requirements. The function to be tested should be precisely known, and quantitative results should be obtained. Neither of these has been exactly fulfilled by the existing methods of functional diagnosis of the heart, first, because the exact nature of the function tested is not given and, second, because the results are not quantitative. In this paper, attention is called to the possibility of elucidating one definite function of the heart by the means of certain graphic methods, phonocardiography and sphygmography. I call these cardiodynamic records. This function, the tonicity, can be considered to depend on the isometric contraction of the myocardium, as expressed in its time element. I will try to bring out some facts in favor of this assumption, after pointing out some well known experimental data on which the hypothesis is based.

THEORETICAL CONSIDERATIONS

Two different forms of contraction occur in all muscle, the isometric and the isotonic. In the first there is no shortening of the individual muscle fibers, but there is a development of internal tension, in the second, actual work is performed, but there is no further increase of the internal tension already developed. In the myocardium, the isometric period of contraction begins with the closure of the atrio-ventricular valves and ends with the opening of the semilunar valves. From the opening of the semilunar valves until the end of the systole, the muscle acts, on ejecting the blood, by its accumulated tension: this is the isotonic or auxotonic period of contraction, rather auxotonic, because the resistance against which the muscle works gradually increases. The isometric contraction or isometric period of muscular contraction, is the essential phase of muscular activity from the point of view of energetics¹. During this isometric contraction, chemical energy of the muscle, partly through the stage of electrical energy, is being transformed into surface energy, creating a state of tension in the fibers². The potential energy of this tension may be transformed into external work. It has been stated that the energy of a contraction is a function of

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1 Hill, A. V. J. *Physiol.* **42** 1, 1911

2 Haber and Klemensiewicz. *Ztschr. f. phys. Chemie* **67** 385, 1909

the area of certain longitudinal surfaces in the fiber ³ these surfaces are being created or increased during the isometric contraction ⁴

Hill proved this point by showing that the heat produced is proportional to the length of the contracting fibers during the time of contraction, and that the heat developed is greatest during the isometric contraction ⁵ Therefore, the work done by the muscle during the isotonic or auxotonic period of contraction depends on this tension, and only after a period of full tension will the work be optimal This tension is created partly during the isometric contraction and is partly pre-existing The latter element of preexisting tension is commonly called

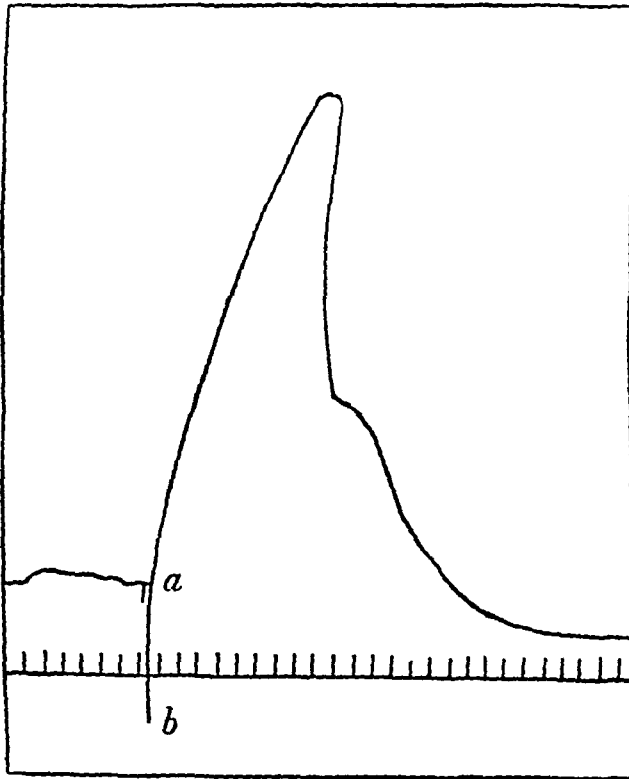


Fig 1—The curve demonstrates the ample contraction of smooth muscle on being stretched from *a* to *b* (From Straub,⁶ Fig 4 B)

muscular tonus, but I believe that it is identical biologically with the surplus tension built up during the isometric contraction

Straub ⁶ and Winkler ⁷ have shown, on the denervated muscle of Lumbricus and the stomach of the frog, that smooth muscle contracts after stretching (Fig 1) The isometric contraction of the myocardium is very similar to this, for, with increased venous inflow the heart is

³ Kozawa J Physiol **49** 233, 1915

⁴ Blin Skandin Arch f Physiol **5** 150 173, 1895 Patterson, Piper and Starling J Physiol **48** 465, 1914 Bawls Principles of General Physiology, Ed 3, London, 1920, p 441

⁵ Hill, A V J Physiol **47** 305, 1913-1914

⁶ Straub Arch f d ges Physiol **79** 379, 1900

⁷ Winkler Arch f d ges Physiol **71** 357, 1898

overloaded and the stretched ventricular muscle performs a greater amount of work, as shown by the increased pressure and volume per beat⁸ It may be said that the law of relation between tension and performance which applies to the skeletal muscle applies also to heart muscle Gesell,⁹ working with the auricle of the tortoise, found that the strength and duration of the total contraction depended on the initial tension³ Thus, it can be said that the preexisting tonus of the heart muscle and the tension formed during the isometric period are identical events, from the functional point of view at least

We may regard another fact as additional proof that the formation of tonus surplus, surface energy or tension is produced during the isometric period of the systole The initial ventricular complex of the

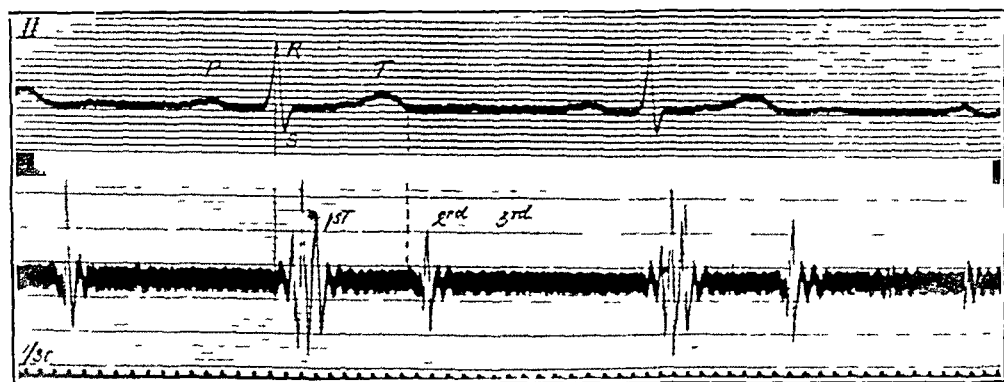


Fig 2—Relationship of systole (expressed in phonocardiogram) to electrocardiogram (after Lewis, from Weitz¹¹)

electrocardiogram always extends considerably into the isometric period The systole begins from 0.023 to 0.035 second after the beginning of the ventricular excitation, and always before the S deflection is inscribed or before the R wave ends¹⁰ Accepting the well established theory of Roaf¹¹ and others, that the production of acid in the muscle is responsible for both the electrical changes and the changes in the surface tension, and observing that the electrical changes in the ventricular muscle fall in the isometric period of the systole, it may be assumed with great probability that the initiation of the changes in surface tension is, too, in the said period¹² (Fig 2)

The principal change recorded in the ventricles during the isometric period of the systole is the rise of intraventricular pressure The curve

8 Straub *Deutsch Arch f klin Med* **116** 409, 1914 Patterson and Starling *J Physiol* **48** 357, 1914 Tigerstedt, R *Physiologie des Kreislaufes*, Berlin, **1**, 1921-1923

9 Gesell *Am J Physiol* **39** 239, 1916

10 Wiggers, C J *Electrocardiogram*, Arch Int Med **20** 93 (July) 1917

11 Roaf *J Physiol* **48** 380, 1914 Mines *Kolloidchem Beihefte* **3** 191, 1912

12 Mines *J Physiol* **44** 25, 1912

recorded by the intraventricular pressure parallels the changes in the internal tension or tonus of the ventricular muscle¹³ It rises suddenly during the isometric period, and maintains itself on a level during the rest of the systole This harmonizes with the previously expressed view Hill¹⁴ demonstrated that the oxygen consumption and the developed tension are directly proportional I have pointed out that the rise in tension and the formed intraventricular pressure are, too, proportional Since the

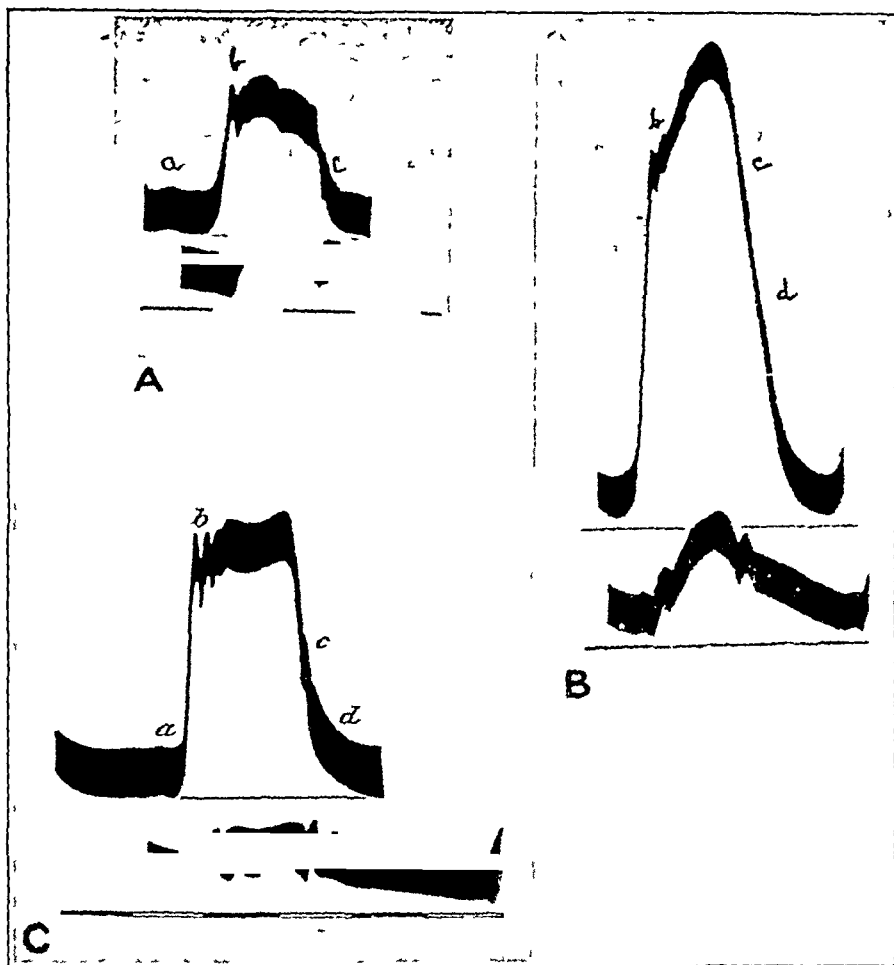


Fig 3—Upper, intraventricular pressure, lower, aortic pressure curves from rabbit *A*, before injection of epinephrin, *B*, immediately after, *C*, after The curves demonstrate the variance in the steepness of the intraventricular pressure according to the resistance encountered (From Tigerstedt¹⁵)

oxygen consumption is a criterion of muscular activity and this means, considered from the standpoint of energetics the formation of tonus then we may conclude that the rise of pressure is the immediate consequence of the tension formed

The function of the intraventricular pressure is to overcome the aortic pressure, open the semilunar valves and impart kinetic energy to the

¹³ De Heer Arch f d ges Physiol **148** 1, 1912

¹⁴ Hill, A V J Physiol **44** 466, 1912 Bavliss (Footnote 4, third reference)

blood This means that the promptness with which the semilunar valves are opened depends on two factors the form and height of the pressure curve itself and the resistance offered to it by the blood pressure in the arterial system But it has already been pointed out by Frank¹⁵ and confirmingly shown by Tigerstedt¹⁶ that the intraventricular pressure rises in the form of a more or less steep curve, according to the height

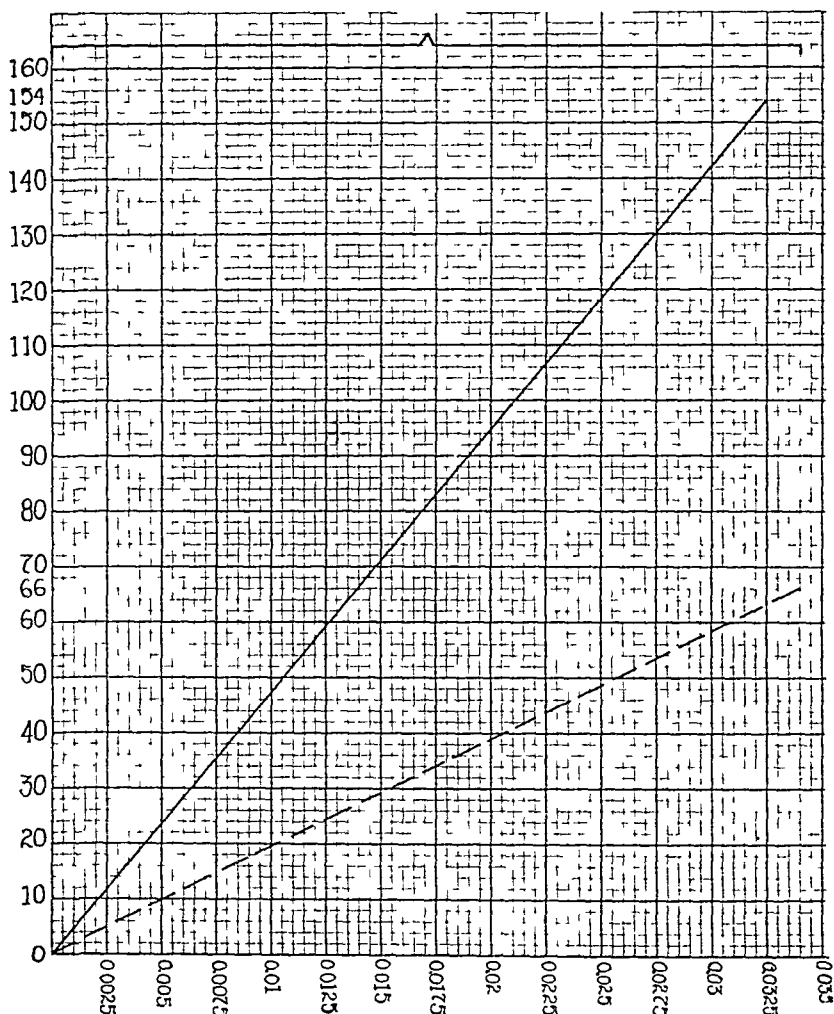


Fig 4—The time element is independent of the absolute amount of intraventricular pressure. Abscissa represents time in seconds, ordinate, pressure in millimeters of mercury (constructed after data from Tigerstedt¹⁶)

which it must attain¹⁷ (Fig 3). If the total height attained was 154 mm of mercury, the pressure rose 10 mm in 0.0024 second, whereas in the case of a total intraventricular pressure of only 68 mm, it rose 10 mm

¹⁵ Frank, O. Sitzungsber. d. Gesellsch. f. Morphol. u. Physiol. in München **14**, 147, 1898.

¹⁶ Tigerstedt, C. Skandin. Arch. f. Physiol. **28**, 37, 1912. Tigerstedt, C. Ibid. **29**, 234, 1913.

¹⁷ Piper. Arch. f. Anat. u. Physiol. (Physiol. Section), 1912, p. 343, *ibid.*, 1913, pp. 331 and 363, *ibid.*, 1914, p. 365.

in 0.0046 second, showing that under normal conditions the tension of the muscle regulates the pressure necessary to open the semilunar valves within constant time limits (Fig. 4). The constancy of the time element is thus entirely independent of the resistance acting against the intra-ventricular pressure, in other words, is independent of the blood pressure.¹⁸

This time limit is given by various authors as varying between 0.02 and 0.1 second.¹⁹ My own values are closer to the lower ones, varying normally between 0.029 and 0.06 second.

I have determined the length of the isometric period by a method similar to that of Wiggers and collaborators.²⁰ As has been shown, the rise of intra-ventricular pressure corresponds to the rise of tension in the muscle fibers. The first sound designates the beginning of systole and of the isometric period, and the second sound the end of systole, which corresponds to the incisure of the central sphygmogram preceding the diastolic wave. If records of the heart sounds and sphygmograms of the carotid are taken simultaneously and the interval between the first and second sounds is marked off on the sphygmogram back from the incisure, the interval during which the atri-ventricular and semilunar valves are closed will be obtained. The rise of the primary wave in the carotid artery designates the beginning of the outflow of blood with the deduction of the average between 0.007 and 0.017 second, this being the time the blood requires to flow from the semilunar valves to the receiving region in the carotid artery.²¹ The oscillations of this factor however do not exceed 10 per cent (plus or minus) of the length of the isometric period and 1.5 per cent of the total systole, and

18 Weitz and Grauer. *Deutsch Arch f klin Med* **116** 512, 1914. Luderitz. *Ztschr f klin Med* **20** 374, 1892.

19 Chauveau and Marcé. *Mem Acad de med* **26** 282, 1863. De Heer (Footnote 13). Edgren. *Skandin Arch f Physiol* **1** 67, 1889. Einthoven and Geluk. *Arch f d ges Physiol* **57** 617, 1894. Grunmach. *Virchows Arch f path Anat* **102** 565, 1885. Hess. *Ergebn d inn Med u Kinderh* **14** 359, 1915. Hochhaus. *Arch f exper Path u Pharmakol* **31** 405, 1893. Hurthle. *Arch f d ges Physiol* **49** 29, 1891. Keyt. *Sphygmography and Cardiography*, New York, 1887. Landois. *Die Lehre vom Arterienpuls*, Berlin, 1872. Marey. *La circulation du sang*, Paris, 1881. Müller and Breuer. *Deutsch Arch f klin Med* **104** 119, 1911. Pezzi. *J de physiol et de path gen* **15** 1178, 1913. Piper (Footnote 17, first reference). Rive. *De Sphymograaf en de sphymographische Kurve*, Utrecht, 1866. Robinson and Draper. *Deutsch Arch f klin Med* **100** 347, 1910. Schmidt. *Ztschr f klin Med* **22** 393, 1893. Swann, A. W., and Janvrin, E. R. P. *Arch Int Med* **12** 117 (Aug) 1913. Tigerstedt (Footnote 8, third reference), *Skandin Arch f Physiol* **20** 249, 1908, (Footnote 16, second reference). Weitz and Grauer (Footnote 18, first reference). Weitz. *Ergebn d inn Med u Kinderh* **22** 402, 1922. Wiggers, C. J., and Clough, H. D. *J Lab & Clin Med* **4** 624 (July) 1919.

20 Wiggers, C. J., and Clough, H. D. *J Lab & Clin Med* **4** 624 (July) 1919. Wiggers, C. J., and Dean, A. L., Jr. *Am J M Sc* **153** 666 (May) 1917. Wiggers, C. J. *Cardiac Murmurs*, *Arch Int Med* **22** 28 (July) 1918.

21 Robinson and Draper. *Deutsch Arch f klin Med* **100** 347, 1910.

are able to influence our results in only a correspondingly slight degree (Fig 5)

Wiggers and Clough²² showed that the isometric period is apt to be prolonged in disease,²³ but Weitz²⁴ and others²⁵ deny this, and maintain that the isometric period is independent of any other value, including the aortic pressure. It is the percental relation between the fixed time interval of the systole and the (physiologically) equally fixed time interval of the isometric period that must be considered. In the isometric contraction, three factors are cooperating, the intraventricular pressure depending on the ventricular tonus, the arterial pressure antagonizing it and the time factor. The time being constant under given conditions,

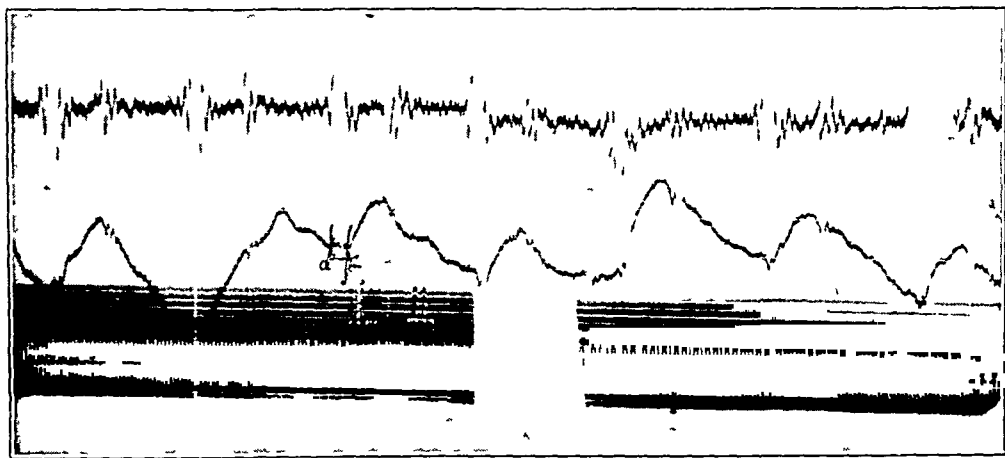


Fig 5—Phonocardiogram and carotid sphygmogram, *a-b*, isometric period of systole, time marked, 0.01 second

it is the tonus that must adapt itself. Normally, this is done, but if the level of the initial tonus is low or if the increase in tension during the isometric period is feeble, the time factor must lose its rigidity in order to enable the intraventricular pressure to overcome the aortic or pulmonic pressure and open the semilunar valves. Thus, the isometric period will be prolonged and its percental relation to the total systole will increase. The average value for the length of the isometric period, as determined from dynamic records, is 16.72 per cent of the entire systole. Changes in the rate alone cause hardly any variation in this percental relation.

ANALYSIS OF RECORDS

One hundred cases have been analyzed by the graphic methods discussed above. They have been divided into four groups, quite

²² Wiggers and Clough (Footnote 19, first reference)

²³ Robinson (Footnote 21) Muller and Breuer *Deutsch Arch f klin Med* **104** 119, 1911. Pezzi (Footnote 19 thirteenth reference)

²⁴ Weitz *Ergebn d inn Med u Kinderh* **22** 402, 1922

²⁵ Hess *Ergebn d inn Med u Kinderh* **14** 359, 1915

arbitrarily, with no clinical or pathologic preconceptions, but solely on the basis of the percental relation between the duration of the isometric period and that of the systole. In the first group have been classified cases the isometric period of which constituted less than 10 per cent of the length of the total systole, to the second belonged cases in which it was from 10 to 15 per cent, in the third group, the values lay between 15 and 20 per cent, and in the fourth group we listed those in which the isometric period was more than 20 per cent of the total systole. Ten per cent of the total material belonged to the first, 28 per cent to the second, 35 per cent to the third, and 27 per cent to the fourth group. Considering the clinical phenomena, we find diverging results in each one of these groups, and it is the last one that shows the most marked differences.

In the first group (ten cases), five were individuals under the age of 17—three of them young children—and the remaining five were under 30. In only three were there clinical symptoms of heart disease. In three of the group, the electrocardiogram showed left ventricular preponderance (in a slight degree, none exceeding an electrical axis of -6 degrees), in one of which this preponderance was attributed to a mild arborization block. None of the members of Group 1 showed clinical signs of decompensation. It seems that values below 10 per cent are frequent in childhood and are not associated with loss of tonicity.

The second group (twenty-eight cases) comprised patients with ages varying from 9 to 54 years. Myocardial disease was demonstrable in three, one showing complete atrioventricular dissociation and two auricular fibrillation, with alterations of the direction of T in more than one lead. Clinically, eight had chronic valvular disease, three were cases of irritable heart (with respiratory arrhythmia and marked cyclic alterations of the electrical axis), four were cases of hyperthyroidism with cardiac symptoms, two others were at the last cases of congenital valvular disease, while eight had no clinical signs of heart affection. All the patients with valvular disease were well compensated. One of the patients with auricular fibrillation (of arteriosclerotic origin) showed symptoms of incipient heart failure, whereas the patient with atrioventricular dissociation was seriously decompensated, with pleural effusion and high values of venous oxygen unsaturation. The electrocardiographic findings of this group included signs of increased auricular activity in three cases, and two mild left ventricular preponderances. Only three, 6 per cent of this group, showed serious myocardial disorder and fifty-three, 58 per cent, had no organic heart lesion.

In the third group (thirty-five cases), six patients showed definite signs of myocardial disease, viz., three, angina pectoris, one, acute myocarditis, one, arteriosclerotic auricular fibrillation, and one, who

exhibited electrocardiographic evidences of disturbed intraventricular conductivity, together with ventricular extrasystoles arising in multiple foci, was accordingly in a state of potential ventricular fibrillation. This constitutes seventeen cases, 14 per cent of the entire group. The fact must be emphasized that, of these six cases, three showed percental values of the isometric period at or beyond the range of normal, namely, 19.93 per cent, 18.46 per cent and 19.69 per cent. These border on the next or pathologic group. Among cases without electrocardiographic evidence of myocardial disease, we find two patients with cardiorenal sclerosis, one of whom showed a percental-value for the isometric period of 19.04 per cent, which again borders on the pathologic. There were ten patients with valvular disease, one, who had mitral stenosis, disclosed signs of clinical decompensation and had 19.34 per cent as the relative value of the isometric period. There was one case of congenital valvular lesion, four cases of irritable heart and one case of hyperthyroidism. The other patients, eleven, evidenced no signs or symptoms of cardiac disease, thus, only 45.41 per cent of this group, the minority of patients, were free from organic heart affection. This percentage however, would increase with the shifting of the five cases with isometric period values approximating 20 per cent to Group 4.

Group 4 comprises cases exhibiting percental values of the isometric period above 20 per cent. In the twenty-seven cases of this group, ten showed definite signs of severe myocardial disease, some with auricular fibrillation developed on an arteriosclerotic or infectious basis, some with well marked symptoms of angina pectoris. We can add to these ten two cases of cardiorenal sclerosis evincing signs of cardiac decompensation, eight cases of chronic endocarditis, three of which were in the stage of heart failure (two being mitral stenosis and one aortic insufficiency), furthermore, one case of congenital heart disease. Thus, twenty-one members of Group 4 (comprising twenty-seven) had definite organic lesions, most of them with signs of cardiac failure. In the remaining six, too, we found many phenomena of cardiac disease, although it was not possible to demonstrate well defined organic lesions. In two there was to be found advanced pulmonary emphysema with strong preponderance of the left ventricle (the electrical axis being between -6 and -35 degrees). In two others, an abnormal auricular activity was encountered, evidenced by ectopic auricular rhythm and frequent auricular extrasystoles, together with marked left ventricular preponderance.

The sixth deserves to be mentioned separately. This was a case of Addison's disease.

The findings are summarized. The age was 14, the blood pressure was 85 systolic, 60 diastolic, there was an inverted T_2 and T_3 in the

electrocardiogram, the electrical axis being 79 degrees, the teleoentgenogram showed a perpendicular heart (Fig 6), the isometric period being 23.5 per cent

These data, taken separately, are indefinite in their meaning, but they harmonize in giving a picture of myocardial hypotonicity, corresponding to the high value of the isometric period. Thus, an involvement of the heart is present in 96.3 per cent of the group, only one case being found to be free from any lesion of the circulatory system. Group 4 is sharply set off from the others, particularly from Groups 1 and 2, Group 4 shows the parallel development of the prolongation of the

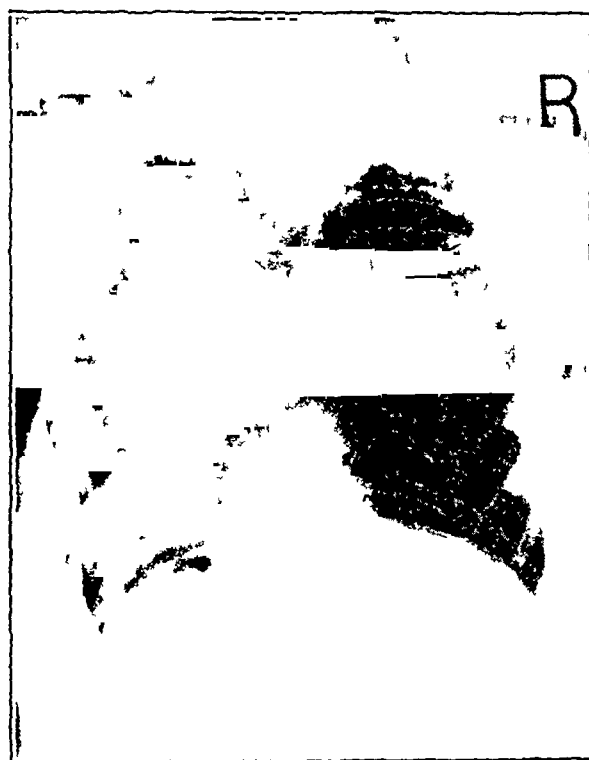


Fig 6—Perpendicular heart in case of Addison's disease, associated with myocardial hypotonicity

isometric period and definite cardiac disease, particularly of arteriosclerotic origin. The accompanying table summarizes the results

Summary of Data

Group	No. of Cases	Relative Length of Isometric Period, Per Cent	Cases with Clinical Cardiac Disease, Per Cent	Age
1	10	<10	33.3	From 2 to 29 years
2	28	10-15	46.4	From 9 to 54 years
3	35	15-20	54.6	From 15 to 65 years
4	27	>20	96.3	From 14 to 62 years

Shifting the five cases with isometric period values approximating 20 per cent from Group 3 to Group 4, we obtain in Column 4 for Group 3, 46.7 per cent, bringing it down to the level of Group 2 whereas for Group 4 we get 96.9 per cent

SUMMARY

1 It is known that the period of muscular contraction, both cross-striated and cardiac, during which the internal tension reaches the degree necessary for the performance of external work is the isometric period

2 The isometric contraction of the myocardium is performed during a given, fixed period of time and is counterbalanced by a given, fixed resistance

3 On this basis, consideration has been given to the idea that in myocardial diseases that involve a decrease in myocardial tonus, the duration of the isometric period in its relation to the duration of the total systole might be disproportionately prolonged

4 The percental relation of the isometric period to the total systole can be regarded as the function of tonicity

5 Its normal value is from 15 to 16 per cent, but there seems to be no definite lower limit. However, the value rarely goes under 13 per cent

6 The upper limit can be set down at 18 per cent. Values above that ought to be considered pathologic

7 The highest values are mostly shown by cases of cardiorenal sclerosis

8 It might be possible in the functional diagnosis of the heart to use the foregoing method which aims at the elucidation of one function, that of tonicity

SKIN CAPILLARIES IN SCLERODERMA *

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Visual exploitation of the skin capillaries by the Lombard¹ method has revealed, in certain diseases, changes in form, size, tonus, flow and reactions to certain stimuli. The study of the skin capillaries in the dermatoses has been reported by Niekau,² Michael³ and others, in which they have noted certain changes in form and function of these vessels. There is as yet no standard established for capillary morphology in normal persons. Sufficient observations have been reported, however, to distinguish definitely from the normal some of the morphologic, numerical and functional variations in the capillaries observed in certain types of cardiovascular-renal disease, polycythemia, Raynaud's disease and related vasomotor states, shock, and a few of the dermatoses. Many of the variations in morphology are only slight. Changes affecting size and number of functioning capillaries, as pointed out by Krogh,⁴ are of greater importance. Pathologic classification should be based on quantitative comparative studies with a large control group in normal persons, and with due consideration of age. These studies should include capillary morphology, size, incidence of tortuosity, flow rates and abnormal types of flow. In this manner, comparisons could be made which would undoubtedly throw additional light on the disturbances of vascular physiology in many different diseases.

The capillary variations observed in cases of primary diffuse scleroderma are striking, but it would not seem justifiable to believe that the capillary changes precede the pathologic hypertrophy of the connective tissue bundles. However, it would appear that a close relationship probably exists between these abnormal vessels and the pathologic changes of the skin. In borderline or vasomotor scleroderma, the capillary studies reveal changes in form and behavior similar to those observed in Raynaud's disease, suggesting that the vascular disturbance

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1 Lombard, W P. The Blood Pressure in the Arterioles, Capillaries and Small Veins of the Human Skin, *Am J Physiol* **29** 335-362, 1911-1912

2 Niekau, Bruno. Anatomische und klinische Beobachtungen mit dem Hautkapillarmikroskop, *Deutsch Arch f klin Med* **132** 301-334 (July) 1920

3 Michael, J C. Dermatoscopy, A Study of the Blood Vessel Form and Arrangement in Various Dermatoses, *Arch Dermat & Syph* **8** 603-618 (Nov) 1923

4 Krogh, August. The Anatomy and Physiology of the Capillaries, New Haven, Conn, Yale University Press, 1922

precedes and stands in direct causal relationship to the secondary change in the skin

Cases presenting skin changes of scleroderma may be divided into three groups (1) the diffuse, primary, progressive scleroderma, (2) borderline, or secondary vasomotor, types, and (3) the localized form, or morphea, which was not included in this study. In this report are included five cases of the diffuse primary scleroderma type, Group 1 (Table 1), and five of the borderline, or vasomotor, type, Group 2 (Table 2). The cases in Group 1 present varying degrees of hide binding, and reduced capillary function. Trophic ulcerations were



Fig 1—Appearance of hands in Case 5, true sclerodactylia

observed in four cases, sclerodactylia in two (Fig 1). Color changes preceded or accompanied the lesions of the skin. They were mild, usually presenting only one phase, in two cases, there was early pallor of the hand, and in two, a mild cyanosis, and in one of these associated facial rubor. In one case, there were no color changes. In Case 5, the earliest symptom was peripheral asphyxia of the hands, aggravated by cold. Syncope or pallor was not observed. Excessive susceptibility to cold, before the recognizable onset of the condition, existed in one instance only, but cold as an exciting or aggravating agent was present in all but Case 3. The hands were affected first, in all cases, the areas were multiple, in five cases. The affected peripheral areas were cold

TABLE 1—Observations on the Capillaries in Diffuse Primary Scleroderma

Case	Description	Age and Sex*	Blood Pressure		Loops of Each Millimeter	Total Length of Visible Capillaries, Mm†	Caliber of Limb, Mm		Capillary Flow, Millimeter Rich Second‡	Observations of Flow	Description of Capillaries of the Nail Fold
			Systolic	Diastolic			Arterial	Venous			
1	Diffuse lesion, mostly on hands and face	50 ♂	Right arm 92	Left arm 70	4	0.58 0.78 0.49			0.30 0.15 0.15 0.30	Slow, uniform flow in all capillaries, no stasis, cells apparently crowding into venous limb	Huge, giant capillaries, dilatation of venous limbs, less marked in arterial limb
2	Diffuse lesion, most marked in hands, small trophic ulcers on tips of fingers, sclerodactylia	31 ♂	100	74	2.3				0.01 Difficult to distinguish flow	Capillary stream slow uniform in all loops, segmented appearance of stream, movement not perceptible in many loops trace of cyanosis in capillary blood	Huge, dilated or giant types of capillaries, few small, thin loops, tortuosity in 50 per cent of the loops
3	Well defined lesions on hands and face	47 ♂	110	70	2.3	0.43			Movement of stream obscured by changes in skin	Difficult to determine movement of stream, skin obscured by trophic changes	Many loops appear as thrombosed areas size not determined, occasional giant type
4	Mixed diffuse boardlike lesions on hands and face	37 ♂	120	60	4	0.40 0.50		0.096	0.128	Obscurity of skin masks movements of capillary flow, few loops show cyanosis	Long dilated, feathery looking loops, few shapeless thrombosed areas
5	Marked boardlike induration on hands, arms and face, sclerodactylia	24 ♀	115	65	3	0.66	0.128	0.016	0.1 0.1 0.014 0.08 0.07	Flow very slow and granular, some intermittence with periods of stasis, no flow disturbance with exposure to cold	Large giant type of loops, averaging three to a field, some obscurity of capillary outline

* In this and the following table, ♂ indicates male, ♀ female

† The visible capillary loop is measured, the transition point from capillary to venule and artery is not always clearly defined

‡ Room temperature, 24 degrees

to touch, this was independent of color. Difficulties of flexion were present in all cases. The molded mask-like expression of the face and restricted ability to open the mouth were noted in three cases.

In the cases in Group 2, the earliest symptoms suggested the abnormal vasomotor reactions observed in Raynaud's disease. In Case 6, the stiffening of the skin was noticed two years after the appearance of the peripheral syncope. The sclerodermatous changes were moderate, and the hands had a swollen appearance, extending up as far as the elbow, but the definite induration observed in Group 1 was absent. Susceptibility to cold with mild peripheral color changes had been present in varying degrees for a number of years. The skin, in four cases in this group, was firm, smooth and unyielding, but not typical of the skin in cases of true scleroderma, changes were noted only in parts presenting the vasomotor phenomena. Minor difficulties in flexion of the hands were present in three cases. Similar involvement in the skin of the feet existed in Case 6. The dystrophic alteration of the skin was more or less transitory, in the cases in this group. A seasonal variation was observed, cold always precipitating or aggravating the condition, and increasing the difficulty of swelling and flexion. There was localized hyperhidrosis in this group, which interfered slightly with accurate visualization of the loops. These cases exhibited, in varying degrees, the well defined color reactions of Raynaud's disease. Pain was a minor feature. The attacks were not clearly defined, and the subjective symptoms were mild, usually of a burning or tingling character.

METHODS OF STUDY

A stereomicroscope was used with magnifications of 60 and 120 diameters. A special ribbon filament light, with condensing lens and suitable heat and color filters, was devised to minimize the effect of heat and color rays on the capillaries. A stabilizer for the fingers, consisting of a small iron trough with rubber buffers, gave sufficient immobilization to assure fairly accurate quantitative studies. Eye piece micrometers with divisions of 0.01 and 0.0025 mm were employed with the low and high magnifications. Photomicrographs were made in several instances, but the difficulties in securing short exposures and mobilization of the part had not been surmounted sufficiently early to allow its routine use in every case⁵. All fingers were examined, and representative areas selected for study. As changes above or below room temperature exert a marked effect on capillary flow, a fairly uniform room temperature was maintained at from 24 to 26 degrees C.

5 Dr. Sheard of the section on physics has recently perfected a method for obtaining photographs of skin capillaries by reflected light in from $\frac{1}{10}$ to $\frac{1}{50}$ second exposures.

TABLE 2—*Observations on the Capillaries in Raynaud's Disease Associated with Sclerodermal Changes in the Skin*

Case	Description	Age and Sex	Blood Pressure		Loops of Visible Capillaries, Mm.	Culber of Lamb		Capillary Flow, Mm. Each Second	Observations of Flow	Description of Capillaries
			Systolic	Diastolic		Arterial	Venous			
6	Mild diffuse lesions of the hands and feet, visomotor disturbances, trophic thickening of index finger	60 ♀	128	84	7	0.70		0.15 0.16	Variation in flow, few loops, slow flow, stasis in others in from two to ten minutes marked slowing response to cold with cyanosis	Ragged dilated loops few thrombosed areas several large types, normal number for each field
7	Mild diffuse sclerodermitous changes, definite Raynaud's syndrome in hands and feet	26 ♀	104	80	6	0.74	0.08	0.15 0.20 0.01 0.03 0.06 0.03	Slow regular flow, few loops show complete stasis, cold produces marked slowing in all loops with stasis, many plasma spaces	Broad dilated loop no giant forms, capillary picture that of Raynaud's disease rather than sclerodermit normal number for each field
8	Waxy, diffuse, firm swelling of hands and feet, color changes suggesting Raynaud's disease	37 ♀	100	74	6	0.44		0.20 0.14 0.12 0.22 0.05 0.07 0.07 0.07	Flow extremely slow furly uniform, greatest delay in venous limb	Broad, dilated loops bizarre shapes, occasional giant type of loop seen, normal number for each field
9	Noninfling swelling of arms hands and feet pale and cold to touch, flexion difficulties trophic area, first finger, right hand	29 ♂			4	0.36	0.08	0.09 0.05 1.1 0.05	Flow difficult to see, slow, not uniform stasis observed	Loops rather broad, hazy appearance, usual, thick, hairpin pattern, decreased number of loops
10	Skin of hands waxy firm thickened some flexion difficulty pale, cyanotic, cold, trophic ulceration, tips of fingers	26 ♀	140	100	5	0.68	0.096 0.128	0.15 0.10 0.12 0.08 0.07 0.06	Intermittent fast and slow flow, plasma spaces and periods of stasis, rocking flow observed, marked stasis reaction to cold	Many huge dilated loops, diminished number in field, few normal sized loops

NORMAL CAPILLARIES

Morphology (Fig 2) —The nail-fold capillaries have been fairly well studied in normal subjects. In subjects under the fifth decade of life, the predominant type of loop is the straight or hairpin pattern, with a small incidence of tortuosity. The percentage of tortuous loops helps to decide the question of normality, since their number increases with age. Tortuosity per se is not of value unless the age of the subject is considered. The decision of the existence of a pathologic condition cannot be made by the capillary morphology unless these changes are definite. Local trauma can disturb the arrangement of the nail-fold

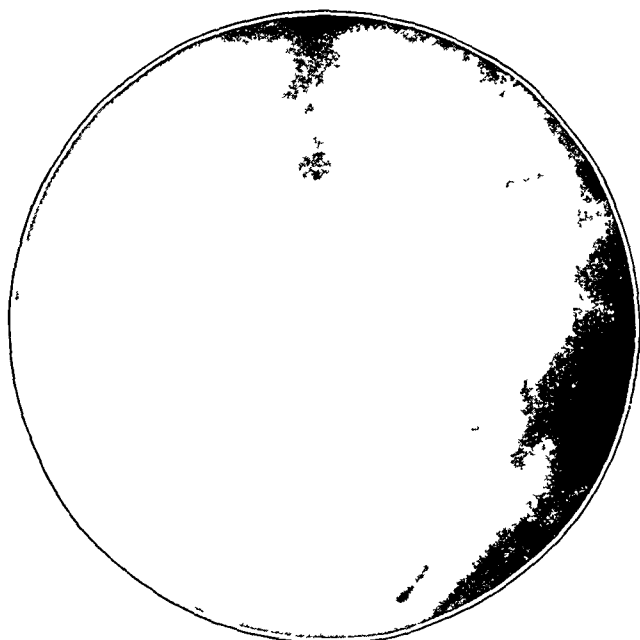


Fig 2—Normal nail-fold capillary ($\times 60$)

capillaries. This is observed as the result of excessive manicuring. Single loops may show marked abnormalities in normal subjects.

Size —The total length of the visible capillary loops has been determined in a group of normal subjects in the different decades of life, and was found to average 0.42 mm. The venous limb is slightly longer and wider than the arterial, the caliber averaging 0.014 mm. The caliber of the arterial limb averages 0.011 mm. The average number of loops for each linear millimeter varies from 7 to 12.⁶

Capillary Flow —The flow is usually fast and fairly uniform. In occasional loops, the stream may be broken or segmented, or, at times,

6 In our previous paper, *The Skin Capillaries in Raynaud's Disease* (Arch Int Med 35:56 [Jan] 1925), the values of the normal calibers in the nail-fold capillaries were too low. Recalculations by means of the photographic and enlargement method have given the values in this paper.

there may be complete disappearance of a loop. The rate of flow varies with age. In the fourth and earlier decades, the majority of loops have flow rates in excess of 15 mm each second, and cannot be measured with accuracy. In later decades, a definite slowing of capillary flow has been demonstrated in individuals with normal range of blood pressure. Temperature modifies the capillary flow markedly.

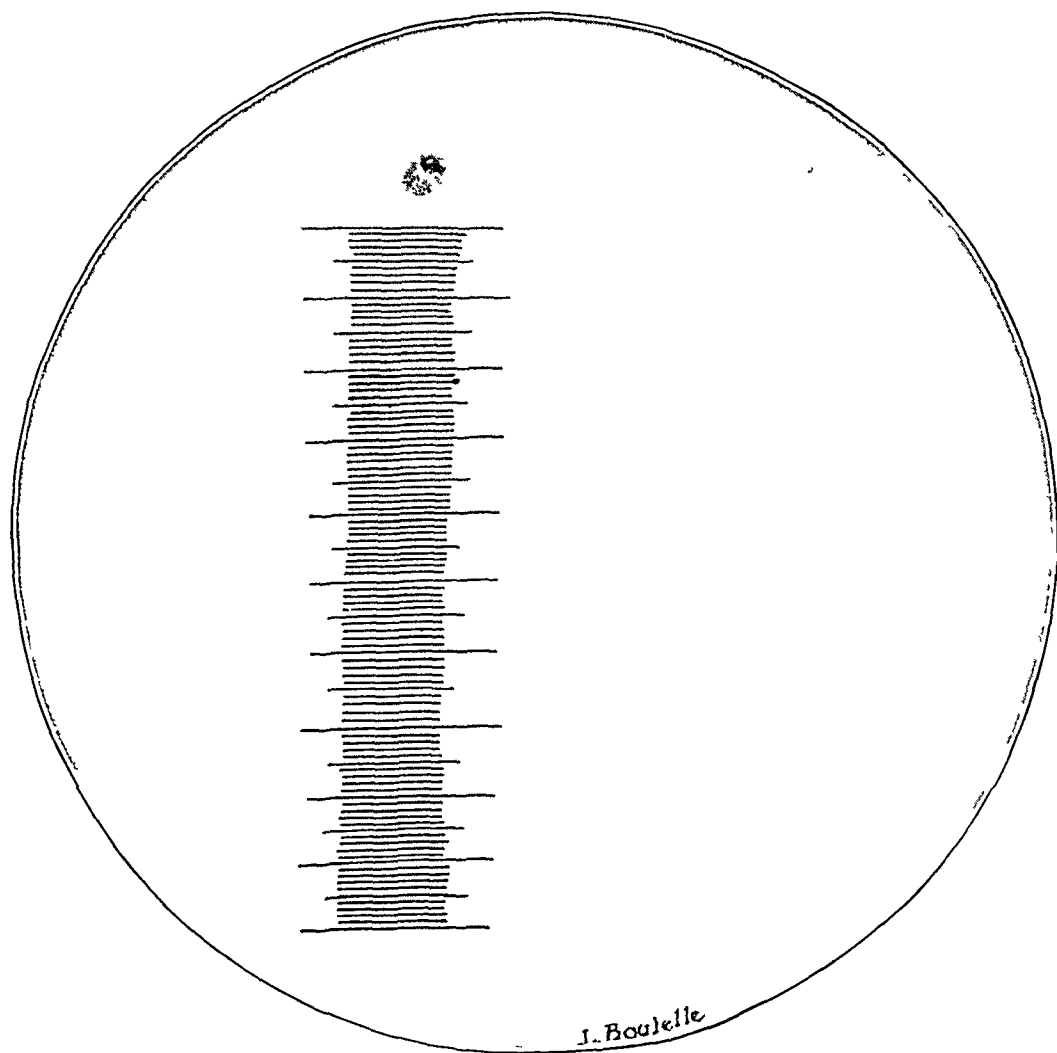


Fig 3—Nail-fold capillaries in case of primary scleroderma, showing marked sparsity of loops and thrombosed appearance, complete loss of normal capillary morphology (artist's sketch drawn to scale, smallest division 0.01 mm)

These estimations were determined under fairly constant conditions with room temperature ranging from 24 to 26 degrees.

Capillary Tonus—The degree of capillary tonus is evidenced by contraction or dilatation in the arterial and venous limbs. Muller⁷ designates contractile changes in the capillaries as spasm and stasis in the

⁷ Muller, L. R. Studien über den Dermagraphismus und dessen diagnostische Bedeutung, Deutsch Ztschr f Nervenhe 47-48 413-434, 1913

precapillary and postcapillary vessels In the normal capillaries, these phenomena are rarely seen under control conditions, and then only in isolated loops In the mild types of acrocyanosis in the asthenic type of person, dilated atonic capillaries are observed In later decades of life, increased tonus is the rule

Capillary Permeability—Direct evidence of disturbances in capillary permeability are occasionally observed, in which free hemoglobin pig-

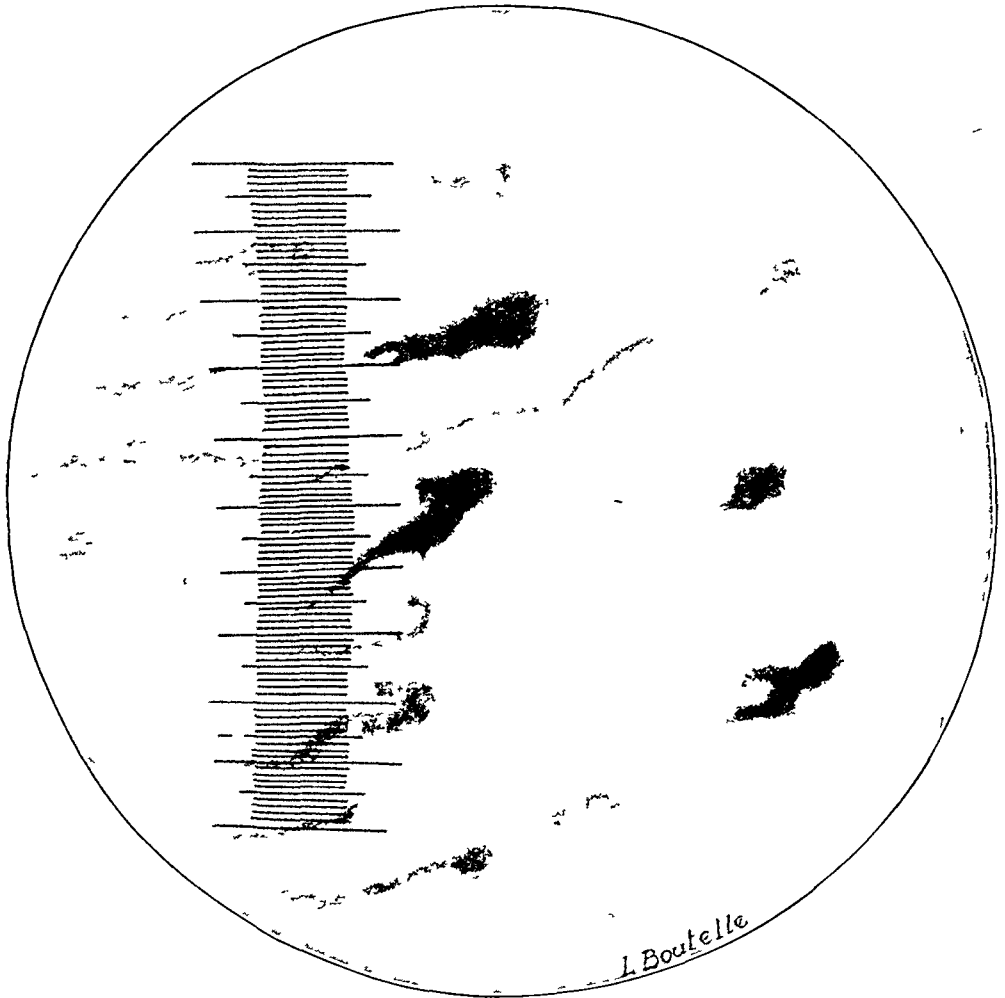


Fig 4 (Case 4)—Loss of normal morphology, huge, dilated and sparse loops (scale same as in Figure 3)

ment is seen distal to the loop with intact capillaries We have observed this, in several instances, in cases of acute glomerular nephritis and in two apparently normal persons, in whom trauma was apparently not a causative factor, since present only in isolated vessels The minor degrees of puffiness of the hands associated with mild rubor or cyanosis and hyperhidrosis are probably related to disturbances in capillary permeability

OBSERVATIONS ON THE CAPILLARIES IN CASES OF PRIMARY
SCLERODERMA

Group 1 (Table 1) —The most striking finding in the skin capillaries, in the cases of true scleroderma, was the presence of huge loops or so-called giant capillaries (Figs 3 and 4) The total length of the larger loops was found to range from 0.5 to 0.8 mm The total number of loops for each linear millimeter averaged 3.5, which is one-third the number observed in normal subjects Frequently, only one or two large capillaries were seen in a field This decreased incidence of loops seemed to stand in direct relationship to the degree of trophic disturbance The diameter of the venous and arterial limbs was much greater than that of normal vessels Often a loop was so greatly enlarged that the space between the two limbs was obliterated, giving the capillary the appearance of a large, red, vaguely defined area These abnormalities were permanent, and transitory changes in the caliber of the loops, as the result of temperature changes and mechanical irritation, were not observed The capillary outline was usually frayed and indistinct, giving the loops a feathery appearance This was due to the decreased visibility of the skin as a result of its collagenous hypertrophy All the capillaries did not exhibit the same degree of morphologic changes Occasional loops appeared normal The venous limb was slightly dilated in most of the loops, and was always less marked in the arterial segment

Capillary Flow —The capillary flow, in this group, was extremely difficult to study The changes in the skin did not allow visualization of the leukocytes, thus making measurements of flow impossible, in most instances The movement of the blood, in the giant loops, was slow, and fairly uniform in different loops Short periods of stasis were observed in Case 5, but marked cyanosis of the capillary blood was not present at room temperature Stasis was not observed in any degree approaching that observed in the vasomotor cases Agglutination phenomena and plasma gaps were not present The blood flow in the capillaries was fast enough to maintain a red color of the cells, that is, a rate exceeding 0.1 mm each second

CAPILLARIES IN VASOMOTOR GROUPS WITH SCLERODERMA

Morphology (Table 2) —In the vasomotor type of case, the capillaries present an entirely different picture which may be useful in differentiating the confused clinical complexes which are now roughly classified as scleroderma The essential difference is shown by the capillaries which resemble in form, size, tonus, function and behavior those observed in true Raynaud's disease Permanent obliteration of the loops is rare The average number of capillaries for each linear

millimeter was six. The large giant loops were observed in only four cases, but the usual capillary pattern was preserved, and the large shapeless loops observed in cases of true scleroderma were not simulated (Fig 5). Decreased tonus with dilated arterial or venous segments was evident, and was heightened by cold. Many loops had the feathery or frayed appearance due to the impaired transparency of the skin, although this was much less than in cases in Group 1. The length of

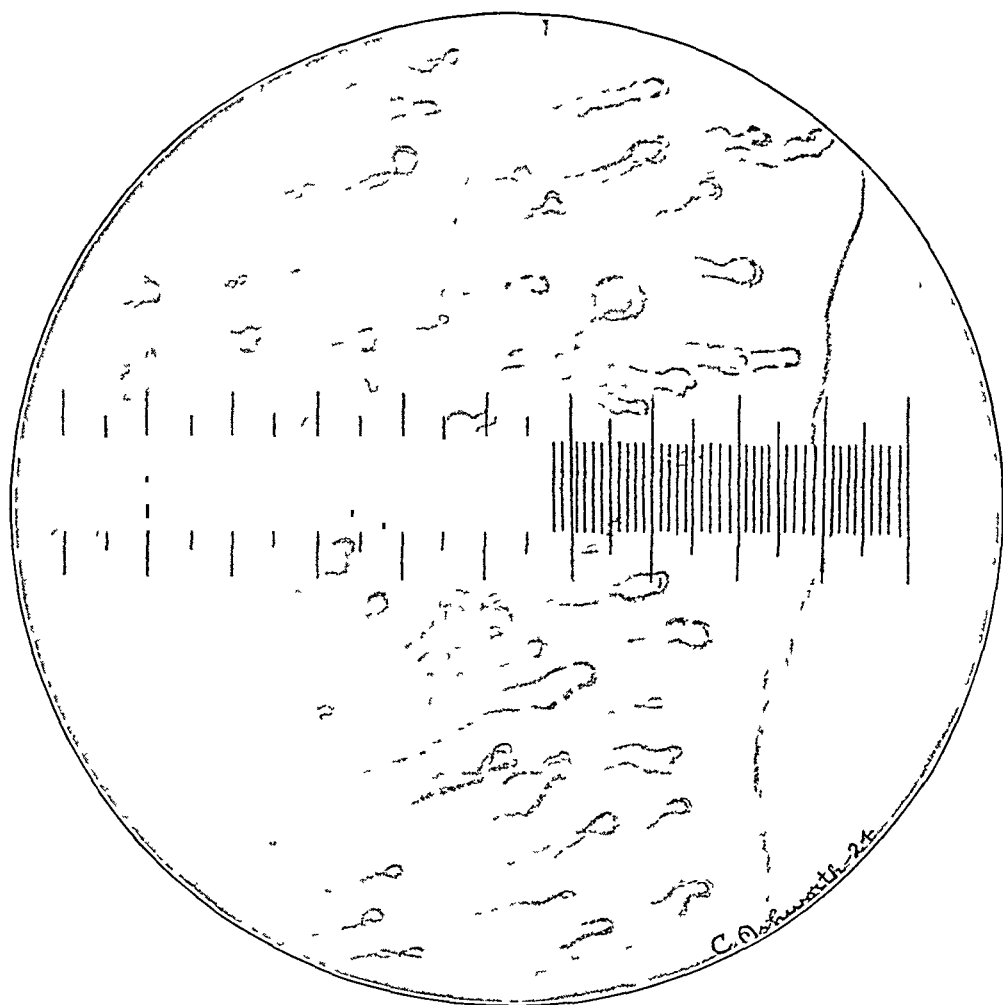


Fig 5 (Case 7)—Raynaud's disease with sclerodermal changes, segmented stream and plasma gaps in many loops. Three dilated loops are visible, but do not approach in size those observed in primary scleroderma, several loops show stasis.

the visible loops was 0.48 mm, which is slightly longer than the average normal visible loop. The caliber of the venous and arterial limb in one case was 0.096 and 0.08 mm, respectively. The incidence of tortuosity was not increased by age.

Capillary Flow—The flow was inert, in many loops, at room temperature. The stasis period varied from a few seconds to several

minutes There was no uniformity of stasis, since contiguous loops exhibited stasis, slow flow or fairly rapid flow, the latter was the rarest Cyanosis was present in the static loops When the capillary flow was less than 0.1 mm each second, cyanosis was recognizable, this point has been designated as the capillary cyanosis threshold The flow was usually segmented, with plasma gaps, and other characteristics observed in true Raynaud's disease⁸ In several instances, the reactions to cold were quite similar to those in Raynaud's disease The behavior of single loops was studied in the different color phases in one case In the pallor or syncope phase, contracted, partially filled loops were present, many loops were invisible In the asphyxia phase, clumps of cells

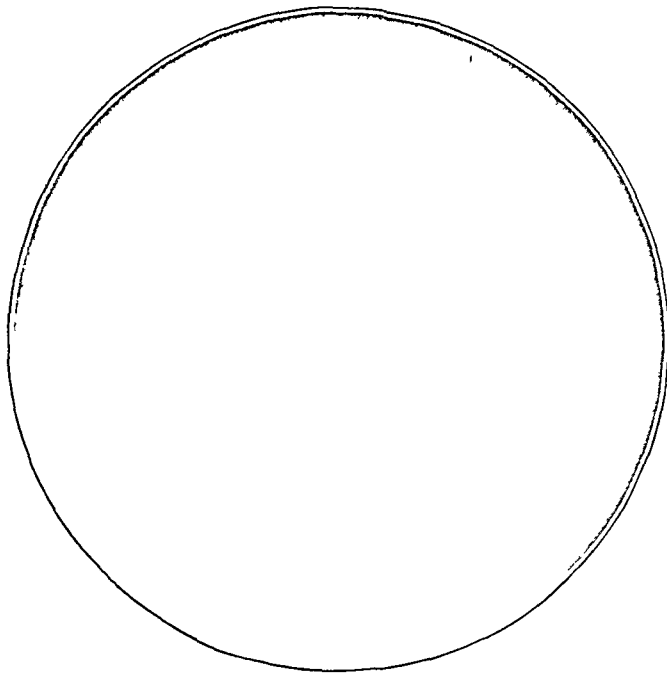


Fig 6 (Case 10)—Capillary in case of Raynaud's disease and secondary scleroderma Only three loops are shown one at right, contracted, one at left, normal, and one dilated loop The flow was inert in all loops ($\times 60$)

were seen to enter the loops slowly from the arterial and venous sides, then complete stasis, increasing cyanosis and dilatation of the loops developed The rubor phase occurs when the flow is resumed with the blood changing to bright red, due to the presence of oxyhemoglobin This stage is often incomplete and, in many loops, there will still be varying degrees of stasis, or alternating periods of fast and inert flow (Fig 6)

COMMENT

The clinical differentiation of certain phases of scleroderma, Raynaud's disease and acrodermatitis chronica atrophicans is still somewhat

⁸ Brown, G E The Skin Capillaries in Raynaud's Disease, Arch Int Med **35** 56 (Jan) 1925

confused. Frequently, vasomotor phenomena, such as cyanosis or mild degrees of peripheral syncope, precede or coexist with the early changes in scleroderma. In the cases presenting the picture of primary or true scleroderma, the vasomotor symptoms are inclined to be mild, presenting only one color phase, unassociated with pain, and not presenting the color responses to cold so characteristic of true Raynaud's disease. This clinical observation is likewise borne out by observations on the capillaries. The numerical decrease in capillary loops was constant in the five cases of Group 1, in contrast to the borderline or vasomotor type of cases in which a fairly normal number of loops were observed. In every case of Group 1, large hypertrophied or so-called giant types were present. The cause of these large vessels might be explained in one of two ways: they may represent a simple capillary hypertrophy, compensating for the decreased number of loops, or they may be a pathologic state related to the fibrotic changes in the corium, that is, back pressure effects. The latter explanation seems more plausible. From the data which we have obtained, we are unable to determine whether the capillaries are a primary or secondary cause of the changes in the skin. The gradual increase in waxy appearance and trophic mutilations accompanying the progression of the disease is associated with single phase color changes, thus indicating capillary impairment. The skin may appear dead and waxlike, or mottled and marmorated. In the more chronic cases, a diffuse pigmentation occurs. The pallor, representing vascular deficiency, is due to a gradual obliteration of many of the capillaries and postcapillary venules. The coldness of the affected portion attests to diminished arterial flow. These vascular changes seem to parallel rather than precede the changes in the skin. It also was observed that the more advanced the sclerodermatous process, the more marked was the capillary deficiency and the incidence of the giant loops. The impaired blood supply of the skin is visualized by the capillary picture, and explains the incidence of trophic lesions in the primary or true type of scleroderma.

The observations on the capillary flow in cases of Group 1 seem to make more definite the distinctions between the primary and borderline or vasomotor types of scleroderma. In the former group, a fairly uniform, slow movement of the cells was observed. Stasis was rare and, when present, was moderate. No intermittent flow, plasma gaps or agglutination phenomena were observed. A crowding of the cells into the venous limb suggested an obstruction in the postcapillary venule. The flow was not slow enough, that is below 0.1 mm each second, to produce definite cyanosis. In the vasomotor group, the morphologic changes in the capillaries were slight, but disturbances of flow were marked. Many of the loops exhibited complete stasis, or alternating

periods of flow and stasis. The flow varied markedly in contiguous loops. Plasma gaps and agglutination processes were common. Cyanosis was present in many of the loops in the compensated stage. The capillary reactions were studied in the different color phases in Case 7. In the syncope phase, there was contraction of capillaries, arterioles and probably venules. In the asphyxia phase, clumps of cells were seen to enter the capillary loops from both the arterioles and venules, indicating incomplete relaxation of these vessels. The capillary flow was inert, and cyanosis increased with increasing dilatation of the loop. After variable periods of time, the flow was resumed with bright red, oxygenated blood. The capillaries thus presented an entirely different picture in the two types of cases. In Group 1 vasomotor phenomena are slight or absent, while in Group 2 these phenomena predominate in the capillary picture. The trophic disturbances, in Group 1, are probably related to quantitative capillary deficiency, whereas, in the vasomotor cases, these disturbances are due to capillary stasis and starvation of tissue, which explains the constancy of manifestations in the skin in primary cases, and the more transitory phenomena in vasomotor cases.

The relationship of the capillary behavior and the disturbances of the skin observed in the vasomotor cases can be explained on the basis of Krogh's experimental work. He found that, when urethan was applied to the capillaries of the tongue of a frog, a marked dilatation occurred with ocular evidence of increased capillary permeability. The cells clumped together with loss of plasma and stasis. Thus, capillary dilatation and stasis represent increased capillary permeability. We could believe that the disturbance in permeability in the vasomotor group, as evidenced by the length of stasis and agglutination phenomena, is sufficient to produce an upset in the tissue-blood equilibrium, involving disturbance of the hydrostatic and osmotic pressures with loss to the capillary blood of water and possibly of colloids. The transitory swelling or puffiness of the skin is clinical evidence of this disturbed equilibrium. We believe that the vasomotor group comprises cases of Raynaud's disease in which the phenomena of water-colloid disturbance has reached a degree to cause changes in the water content of the skin, simulating those of scleroderma. Practically all cases of Raynaud's disease reveal some evidence of metabolic disturbance of the skin. This may be represented merely by excessive localized sweating, cold, or by minor degrees of puffiness or swelling. When these alterations exist in sufficient degree, simulation of true scleroderma occurs.

The histopathologic picture of the clinically recognizable scleroderma is that of an increase in connective tissue elements in the corium and subcutaneous tissue, with a diminution in the caliber of the vessels, and thickening of the media and the adventitia, proceeding to atrophy from

the sclerosis, and pressure. However, in the early cases of scleroderma in which the foregoing processes have not as yet occurred, the value of a biopsy becomes limited. The use of histopathologic study in the Raynaud syndrome does not, as a rule, permit of definite deductions. Therefore, the taking of a biopsy in these borderline cases will not help materially in the differentiation.

Although a successful treatment is not available in either group of cases, the differentiation of these types is of more than academic interest, since the pathologic basis for a rational treatment would differ diametrically in both groups.

SUMMARY

The nail-fold skin capillaries were studied quantitatively in a series of ten cases, five of which presented a definite picture of scleroderma, and the remaining five essentially a Raynaud syndrome with associated sclerodermatous changes. From the difference in capillary morphology, size, tonus and flow, the cases were divided into two groups. Group 1, true diffuse scleroderma, in which there was a quantitative deficiency of capillary loops, which roughly paralleled the trophic disturbances; and Group 2, the borderline or vasomotor type, in which the skin disturbances were apparently secondary to the abnormal vasomotor reactions, with dilatation, stasis and permeability disturbances of the capillaries. These cases are apparently true Raynaud's disease, the changes in the skin attaining a degree to cause clinical confusion with true scleroderma.

It is apparent that the careful study of the skin capillaries will assist in the differentiation of these cases, but that further study of the primary group is necessary before it will be possible to make any deductions as to the etiologic rôle played by the capillaries.

REPORT OF CASES

CASE 1—A Canadian veterinarian, aged 50, had had a septic infection in 1910. The scleroderma began three years before the patient came to the Mayo Clinic. While driving a car in extremely cold weather, he noticed numbness and pallor of the hands, which persisted in varying degrees. Flexion movements became impaired, and the skin gradually became stretched and shiny. The symptoms were aggravated by cold, but there were no color changes suggesting Raynaud's disease.

At the time of the examination, the hands were cyanotic and cold, the skin was hard and glazed, but not tender. The feet were bluish pink and marmorated. The peripheral arteries pulsated normally. A masklike appearance of the face and partial immobilization of the lip were noted early.

CASE 2—An English farmer, aged 31, first noticed scleroderma during a period of extreme cold three years before coming to the clinic. The first and second fingers of both hands were tender to touch, and it was thought that they were frozen. The toes and ears were similarly affected, and became cyanotic in about three months. There was gradual stiffening of the fingers, followed by pain on motion. The skin became dry and shiny, and, during the last year, the

skin of the face had also become red, hard and shiny. Necrotic areas appeared over the knuckles and elbows and the tips of the fingers.

At the time of examination, the hands and feet were moderately cyanotic, and cold to touch. The skin was tense, hard and shiny, with small dry ulcers on the elbows, and on the middle joint of the third fingers. There was limitation of motion of the fingers and elbows. The skin of the face was firm and had a varnished appearance.

CASE 3—A railroad man, aged 47, an American, had had multiple infections, rheumatic fever, typhoid, influenza, malaria and possibly syphilis. The onset of scleroderma was one year before the patient came to the clinic, when the hands swelled slightly during the summer. Three months later, small ulcers developed on the tips of the fingers, with increasing stiffness of the hand and hardening of the skin. There was slight burning and increased sensitivity of the skin. The process had extended gradually.

At the time of examination, there were well defined, hard, diffuse, sclerodermatous lesions of the hands, feet and arms, anterior chest and abdomen, face and neck. There was no definite pigmentation, and there were no color changes. Motion was impaired 20 per cent. Small trophic areas were present on the tips of the fingers.

CASE 4—A mining engineer, aged 28, an American, first noticed scleroderma during exposure to cold weather two years before coming to the clinic. The skin of the finger tips cracked, the fingers became pale, and, when warmed, were slightly cyanotic. The condition continued with mild color changes when he was exposed to cold, and there was a tendency to cracking of the skin. Six months ago, the hands, ankles and knees began to swell, and since then the swelling has spread to the forearms and legs. These extremities were painful on motion, and the patient felt cold. There was hard swelling in the face, and gradual stiffening of the skin of the hands and face.

At the time of examination, the face was masklike, and the skin hard and shiny. The hands were cyanotic, swollen, hard and cold to touch, the atrophic changes were not marked. Small trophic ulcers of the finger tips were noted. The lesion extended above the elbows, and there was a similar condition in the feet extending to the knees.

CASE 5—An Italian woman, aged 24, had had pleurisy in 1923, and a diminution in menstrual flow for the last three years. The scleroderma began suddenly, twelve years before the patient came to the clinic, with symmetrical asphyxia of the fingers to the first joint. There was no preceding pallor. The condition appeared after exposure to severe cold, and was followed by redness with a burning sensation. The following year, keratoses appeared on the finger tips. During the last five years, the skin had become shiny and stiff, gradually involving the wrists. There was dry necrosis, loss of the terminal joints of the fingers, and increasing general pigmentation, more marked over pressure areas. The skin of the lips, nose and shoulders had become tighter, causing difficulty in movement.

At the time of examination, the skin of the hands and face was hard, glistening, shiny and stretched in appearance. Atrophy was marked. There was some destruction of the terminal phalanges, trophic ulcers of the malleolar and olecranon areas (Fig 1), and general brownish pigmentation, most marked along spinous processes.

CASE 6—A housekeeper, aged 60, an American, four years before coming to the clinic had had blanching and cyanosis of the fingers to the distal joints, following exposure to cold. There was no pain during the period of recovery, merely a mild sensation of burning. During the last two years, the susceptibility to cold had been more marked. The hands and feet had become puffy and felt uncomfortable, and flexion of the hands was slightly impaired.

At the time of examination, the hands were red, swollen and slightly shiny. Flexion was incomplete. Slight induration extended to the elbows. Venipuncture revealed definite hardening of the skin. Small, dried, necrotic areas

in the tips of the fingers were found Exposure to cold caused pallor and cyanosis, followed by the rubor and a burning sensation The peripheral vessels were palpable with normal pulsation, and the blood pressure was 138 systolic, and 84 diastolic

CASE 7—A telephone operator, aged 26, an American, first noticed color disturbances in the hands six months before coming to the clinic, during a nervous breakdown from overwork Two months later, she noticed that the hands turned white on exposure to cold, and that the feet and hands became swollen The skin was shiny, and there was difficulty in flexion Cyanosis would follow blanching spells, and then redness with burning, and a disagreeable sensation similar to chilblains Two months later, pain developed during the period of syncope

On examination, the skin of the hands was found to be shiny and glazed in appearance The skin of the feet was slightly firm to touch There was about 75 per cent flexion in the hands No atrophic changes in the skin were noted At room temperature, the hands were red and cool With colder temperatures, the fingers became mottled with pallor and cyanosis The peripheral vessels were palpable with normal pulsation

CASE 8—An American housewife, aged 27, had a nervous breakdown on the day of her husband's death, three years before coming to the clinic Her voice became hoarse, and she could not speak above a whisper Swelling appeared in the fingers, with a dead, numb feeling, and, on exposure to cold, the second finger of the left hand turned white to the first joint There was no pain Syncope attacks continued, and affected all the fingers during cold or wet weather, but not the thumbs Later, cyanosis followed the blanching, then redness with deep seated soreness, which felt as if it were in the bone The skin had become firmer, and complete flexion of the hands was not possible The patient complained of a sensation of full distention in the hands, and a similar, but more marked, sensation in the feet

At the time of examination, the skin of the hands was shiny and felt slightly stiff, but was not boardlike or dense There was no atrophy, but the hands were somewhat cyanotic and cold to touch They became slightly pale with temperature changes, then presented a bluish, mottled appearance, followed by irregular distribution of red areas The color changes were not complete The peripheral vessels were palpable with weak pulsation

CASE 9—A man, aged 29, an American, had scleroderma which began after a severe shock in a railroad accident The patient first noticed loss of sensation in one finger on exposure to cold, followed by minor degrees of cyanosis and rubor This condition persisted, but without pain Two years later, the hands and feet became swollen and tense Color changes were more pronounced, and varied with cold and exercise Recently, the hands and feet had become pale and cold, and color changes became more marked with lesser degrees of cold It was difficult to flex the hands, and the fingers were swollen and rather firm There was no atrophy A dry trophic area was noted on the right thumb A mild tingling and numb sensation accompanied exposure to cold

CASE 10—An American housewife, aged 26, had always noticed blanching of the fingers on exposure to cold Ten months before coming to the clinic, she noticed a transitory cyanosis of the finger tips, extending to the distal joint, the attacks lasted about fifteen minutes There was no distinct pain, but numbness and color changes were noted The condition was aggravated by cold, being particularly bad during cold damp weather For the last three months, the patient had had transitory edema of the face No trouble had been noticed in the feet

Examination revealed the facial lesion to be disseminated lupus erythematosus There were small trophic changes in the tips of the fingers, with slight loss of tissue and small, dried ulcerations The skin of the hands was firm and slightly thick There was no atrophy of the skin, which was pale and cyanotic

AN ACUTE FEBRILE PLEIOCHROMIC ANEMIA WITH HYALINE THROMBOSIS OF THE TERMINAL ARTERIOLES AND CAPILLARIES

AN UNDESCRIBED DISEASE¹

ELI MOSCHCOWITZ, M D
NEW YORK

This case is remarkable, clinically and anatomically

REPORT OF CASE

History—K Z, a girl, aged 16 years, was an elementary school graduate, had gone to business school, and had been employed for eight months preceding the illness. There were three other children, two younger and one older, all apparently were perfectly normal. There were no home difficulties, and poverty was not extreme. She had spent September 4 and 5 at Rockaway Beach, where she appeared in perfect health and spirits. She had returned home on the evening of September 5 and slept well. On the morning of September 6, she complained of weakness in the upper extremities and had pain on moving the wrists and elbows, she already had marked pallor and was slightly constipated. The symptoms increased in severity until she was admitted to the Beth Israel Hospital, September 15. While at home, she had a constant fever, the temperature rising once to 104 F and staying at other times between 101 and 102 F.

Physical Examination—The patient was a pale girl with "café au lait" tinge. A few petechiae were present on the left arm. The lungs and heart revealed nothing abnormal. The spleen and liver were not enlarged. The abdomen was lax and not tender. September 18, the red blood count was 1,330,000, the hemoglobin, 40 per cent, the leukocytes, 12,600, of which 65 per cent were polymorphonuclears. The red cells revealed a central pallor, but there were no nucleated elements. A fragility test showed hemolysis to begin at 0.8, and to be complete at 0.19 (?). No platelet count was made. September 19, the red blood count was 1,120,000, the hemoglobin was 40 per cent, and the leukocytes were 19,000. A blood culture remained sterile.

The urine showed marked traces of albumin with hyaline and granular casts.

The blood chemistry, September 16, showed urea, 21.2 mg per hundred cubic centimeters, nonprotein nitrogen, 31.25 mg per hundred cubic centimeters, and creatinin, 1.1 mg per hundred cubic centimeters. The feces and gastric contents gave a marked reaction for occult blood.

Röntgen-ray examination of the chest showed nothing abnormal. The electrocardiogram showed inversion of the T wave in Lead III. The temperature, on admission, was 101.8 F. During the week the patient was in the hospital, it ranged between 100 and 102 F. The pulse varied between 100 and 130. The respiration was around 20. The systolic blood pressure was 130, and the diastolic, 60.

September 19, there was partial paresis of the left arm and leg, also, a slight facial paralysis. The following day, a double Kernig reflex was noted. That night there was pulmonary edema, which responded to treatment. Soon after, the patient went into coma, respirations became irregular, and she died, September 20. Dr E. Libman, who saw this patient in consultation, recognized the condition as a new disease.

¹ Read before the New York Pathological Society, Feb 7, 1924.

Necropsy—A partial necropsy was done. The body was pale and poorly nourished. The lower lobes of both lungs showed marked congestion. The heart was slightly enlarged, the left ventricle was hypertrophied, the muscle was firm and pale. The mitral and aortic valves were normal. The liver was slightly enlarged, pale and fatty, there was slight nutmeg change. The spleen measured 11 by 8 by 3 cm, and weighed 165 gm. The surface was smooth. On section, the organ was deep mahogany red, somewhat soft and velvety. The malpighian bodies were prominent. The kidneys were large, the capsules were smooth and not adherent, on section, the organ was deep red.

The anatomical diagnosis was anemia, acute congestion at the bases of both lungs, hypertrophy of the left ventricle of the heart, hyperplasia of the spleen, and congestion of the liver and the kidneys.

Microscopic Examination—The lungs were edematous, at the bases, there was congestion of the parenchyma. The heart muscle revealed a striking

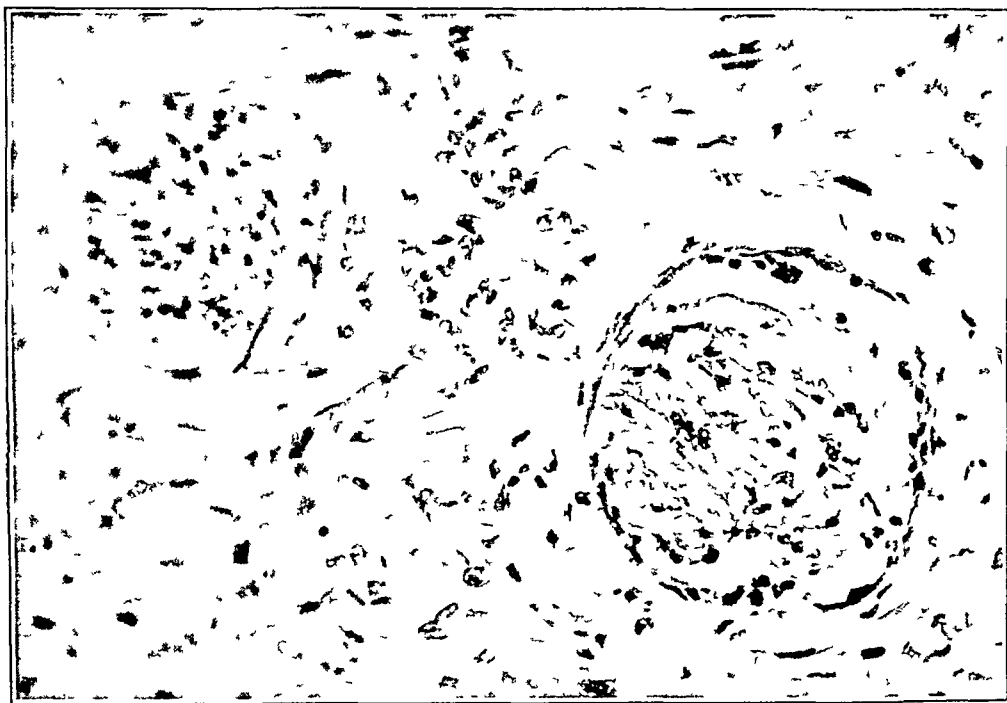


Fig 1—Section of heart muscle, showing hyaline thrombi in early stage

appearance. With the low power of the microscope, practically every field revealed from one to a dozen structures that were unquestionably thrombi in the terminal arterioles or capillaries. These varied in appearance and revealed progressive changes depending on the amount of organization that had taken place. The earliest (Fig 1) showed merely a plugging of the vessel with a hyaline mass which either partially or completely filled the lumen. Usually, even in this stage, the plug, if not in intimate contact with the wall of the vessel, was surrounded by a layer of flat cells of the fibroblastic type which was distinct from the endothelial intima. In older plugs (Figs 2 and 3), fibroblasts penetrated into the hyaline mass, and the older the plug, the greater became the amount of fibroblastic infiltration at the expense of the hyaline material, eventually, a small fibroblastic tubercle-like structure was formed. In some of these thrombi, the origin of these fibroblasts from the endothelium of the vessel was plainly discernible. At the same time, the process of organization within the lumen was accompanied by a fibroblastic process around the wall of the vessel in concentric fashion, with the van Gieson stain, some gave the reaction for fibrous tissue. Karyo-

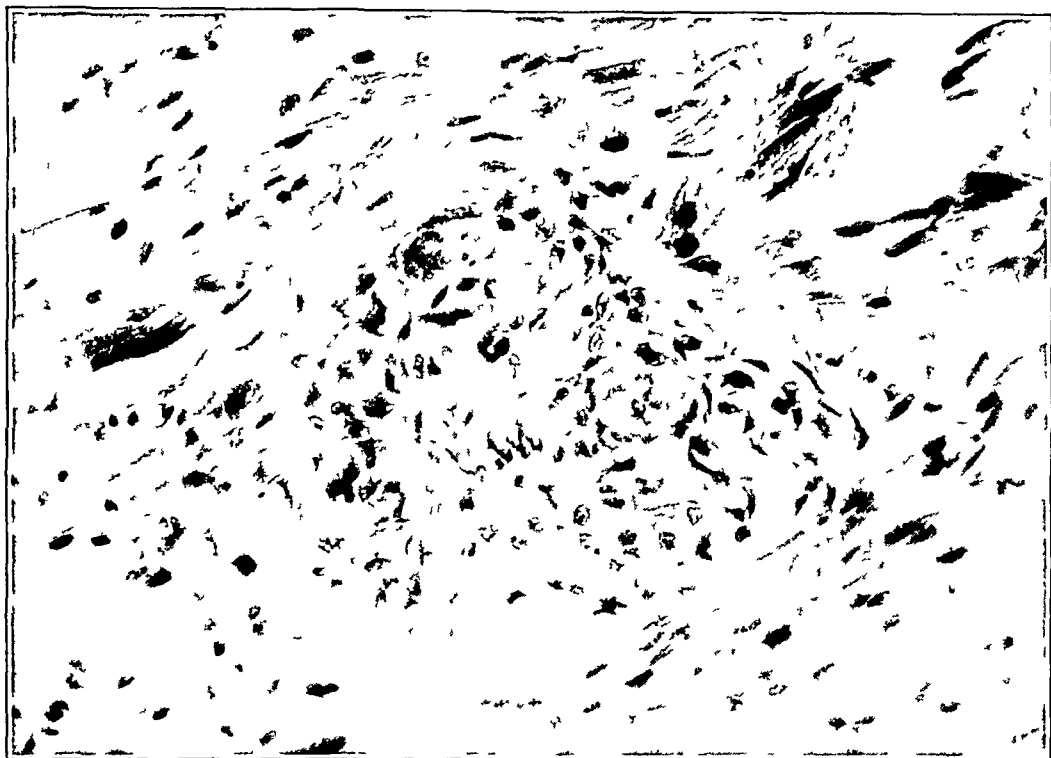


Fig 2—Section of heart muscle, showing hyaline thrombi with beginning organization

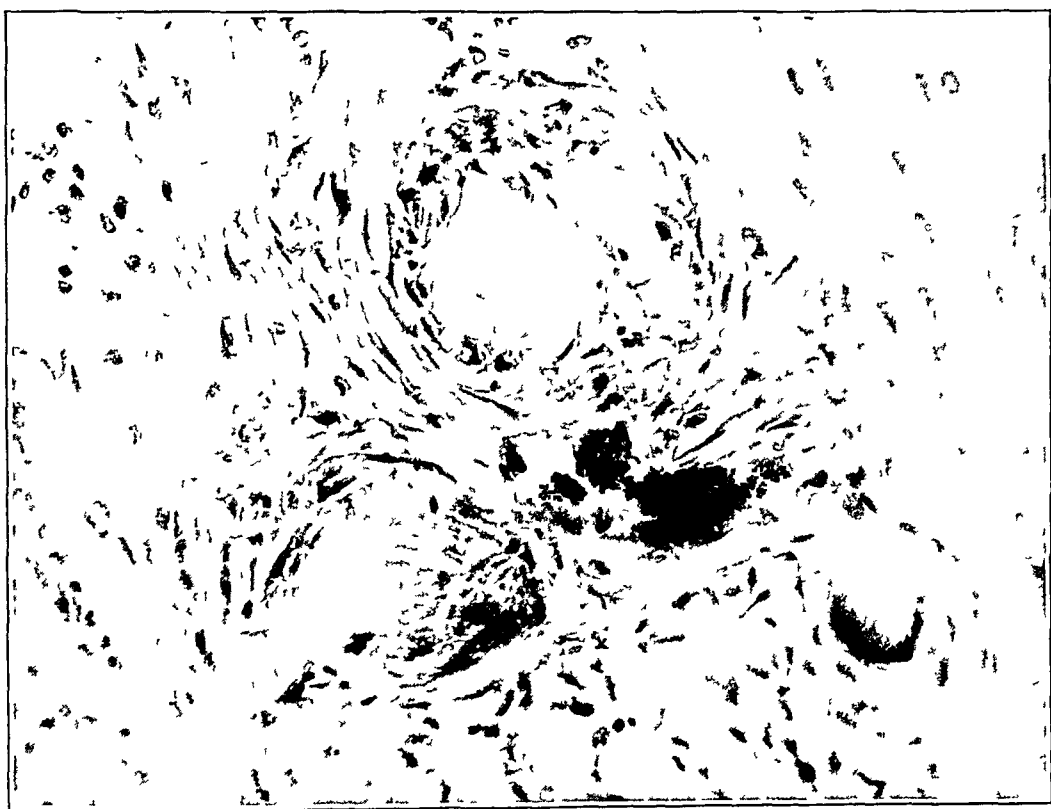


Fig 3—Section of heart muscle, showing hyaline thrombi with beginning organization

kinetic figures in these fibroblasts were common. In some vessels in which the plug had not completely filled the lumen, tiny spaces were formed in which fresh red cells were visible. Only the terminal arterioles and capillaries were involved. The larger vessels with well defined muscular walls showed no change whatever, either in the form of thrombosis or changes in the intima. Every section of heart muscle was involved, the ventricles, the auricles, the papillary muscles and the septum, they were even visible in some of the vessels of the precordial fat. In addition, there was moderate edema of the parenchyma. The liver showed a moderate fatty infiltration and slight congestion around the central veins. Very few hyaline thrombi in the early stages were noted. The spleen showed enormous congestion of the sinuses. A few hyaline thrombi were present in some of the central vessels of the malpighian bodies. The kidneys showed marked parenchymatous degeneration in the tubules and congestion of the parenchyma. The malpighian tufts were

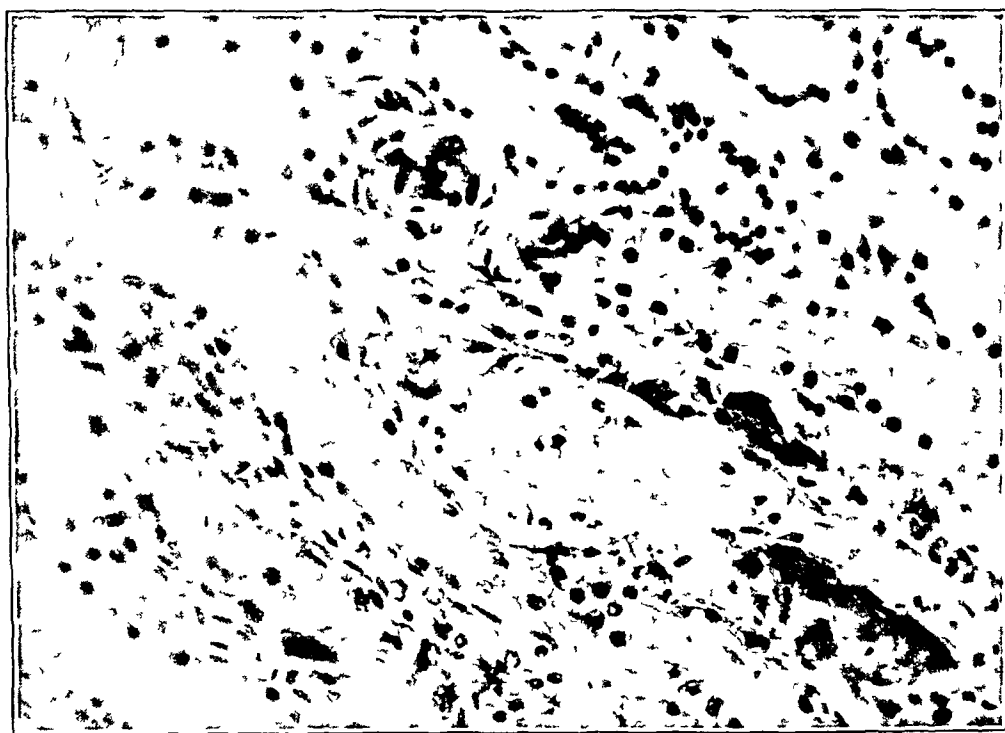


Fig 4—Section of kidney, showing hyaline thrombi in terminal arterioles

clear. Many of the arterioles and capillaries in the middle zone, in the region of the vasa recta, showed hyaline thrombi of the same morphology as those in the heart (Fig 4). No bacteria, tubercle bacilli or spirochetes were found in any of the tissues.

A fairly complete search of the medical literature fails to reveal a case resembling this, either clinically or anatomically. Hyaline thrombi have long been recognized but never in respect to the enormous spread and distribution revealed in this case. Klebs¹ apparently was the first to describe these thrombi, he noted them in cases of extensive burns. They have been described in a wide variety of conditions. Thus, Kaufman² found capillary red cell thrombi in cases of mercury poison-

1 Klebs. Handb d path Anat Berlin, 2 114, 1868-1880

2 Kaufman, quoted by Dietreich (Footnote 6)

ing Schmorl³ found them in the stomach and liver after abrin poisoning Flexner⁴ noted such thrombi in the neighborhood of ulcerated typhoid ulcers of the ileum and in the lung, associated with bronchiectasis, also in the liver in enclampsia, and in the stomach after carbolic acid poisoning He also states that they have been found in pneumonia, diphtheria and in some of the acute infectious diseases Loeb, Stricker and Tuttle⁵ noted red cell thrombi in the lungs of animals after the injection of a foreign serum Dietreich⁶ found such thrombi in animals after the injection of an extract from beans Bacterial infections are common causes of such thrombi, for they have been produced experimentally, in infections, by the hog cholera bacillus (Boxmeyer⁷), the pneumococcus and the staphylococcus aureus

The true pathogenesis of hyaline thrombi was not known until Flexner showed that they arose from agglutinated red cells This he proved by injecting the highly agglutinative substance, ricin, into rabbits He concludes that when red cell thrombi are old or when agglutination is compact, they have the appearance of hyaline thrombi He also states that poisons that destroy red blood corpuscles rapidly provoke agglutinative thrombi, and that the so-called fibrin ferment thrombi are probably agglutinative thrombi Pearce,⁸ indeed, obtained hyaline thrombi in the liver associated with focal necroses by injecting agglutinating serums He also injected filtered autolyzed products of various bacteria which possess hemagglutinins of low activity and obtained similar necroses of the liver associated with fused red blood cell thrombi, i e, structures resembling hyaline thrombi

In this condition, it is interesting to note that, as far back as 1875, Landois⁹ found that an injection of foreign blood causes multiple capillary thrombosis

I have learned that Dr Max Lederer of Brooklyn has seen four cases clinically identical with the one described in this report He permits me to state that, thus far, no cause has been found, and that all four patients recovered promptly after a single transfusion of blood

From these observations we conclude that death, in the case described, resulted from some powerful poison which had both agglutinative and hemolytic properties If opportunity offers, further investigation will be made of this strange disease with strange pathologic morphology

3 Schmorl Jahresb d Gessellsch f Nat-u Heilk in Dresd, 1899-1900, quoted by Flexner (Footnote 4)

4 Flexner J M Res **8** 316, 1902

5 Loeb, Stricker and Tuttle Virchows Arch f path Anat **201** 5, 1910

6 Dietreich Centralbl f Path **23** 372, 1912

7 Boxmeyer J M Res **9** 146, 1903

8 Pearce and Winne Am J M Sc **128** 669, 1904

9 Landois Die Tranfusion des Blutes, Leipzig, 1875, p 225

THE VITAL CAPACITY IN ARTIFICIAL PNEUMOTHORAX

THE MECHANISM AND THE FACTORS MODIFYING THE VITAL
CAPACITY, WITH ESPECIAL REFERENCE TO ITS
CLINICAL AND PROGNOSTIC VALUE IN
THE COLLAPSE THERAPY *

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That a certain relation exists between the degree of structural involvement of the lung tissue and the diminution in the vital capacity has been known since Hutchinson¹ published his observations on the vital capacity in a great variety of subjects, but that the recuperation of pulmonary function seems to be possible even when the integrity of the lungs is not perfect is of great clinical importance and deserves more extensive investigation. The various functional modifications of the respiratory capacity taking place during the entire course of therapeutic pneumothorax afford a great deal of information in this respect.

A priori one would expect a quantitative relation between the amount of air introduced into the pleural cavity and the diminution of the vital capacity. Such, however, was not the case in many of our tuberculous cases treated with artificial pneumothorax, the same quantity of air introduced into the pleural cavity produced different results in the reduction of the vital capacity not only in different cases, but even in the same case at various insufflations. It follows that the amount of air introduced into the pleural cavity is not the sole factor affecting the decrease in the vital capacity, but many other factors come into play. It varies with the type of pneumothorax with the functional and pathologic status of the treated lung, as well as the condition of the contralateral lung, with the movements of the diaphragm and mediastinum and with the cardiac ability to compensate. Each of these factors has a definite bearing on the vital capacity and may affect it jointly or singly.

It was the purpose of these experiments to find out which of the factors plays a more important rôle in the reduction of the respiratory capacity what clinical assistance we can derive from the periodic vital capacity measurements as to the amount of gas to be administered at each insufflation and how much spirometry can serve as a prognostic criterion in the collapse therapy.

* From the Montefiore Hospital, Bedford Sanatorium for Incipient Tuberculosis

1 Hutchinson, J. On the Capacity of the Lungs and on the Respiratory Functions with a View of Establishing a Precise Method of Detecting Disease by the Spirometer, *Med-Chir Tr* 29 137, 1846

MODIFICATION OF VITAL CAPACITY IN VARIOUS TYPES OF
ARTIFICIAL PNEUMOTHORAX

In considering the vital capacity changes that take place with each injection of air into the pleural cavity, the distinction between the types of pneumothorax existing at the time of insufflation must be kept clearly in mind. In a previous article,² I indicated that at least three distinct types of induced pneumothorax exist with reference to the undiseased portion of the lung.

(a) *Expansile pneumothorax*, in which the intrapleural pressure is much smaller than the intrapulmonary pressure and the lung is not compressed, but collapsed or retracted by virtue of its own elasticity, and still

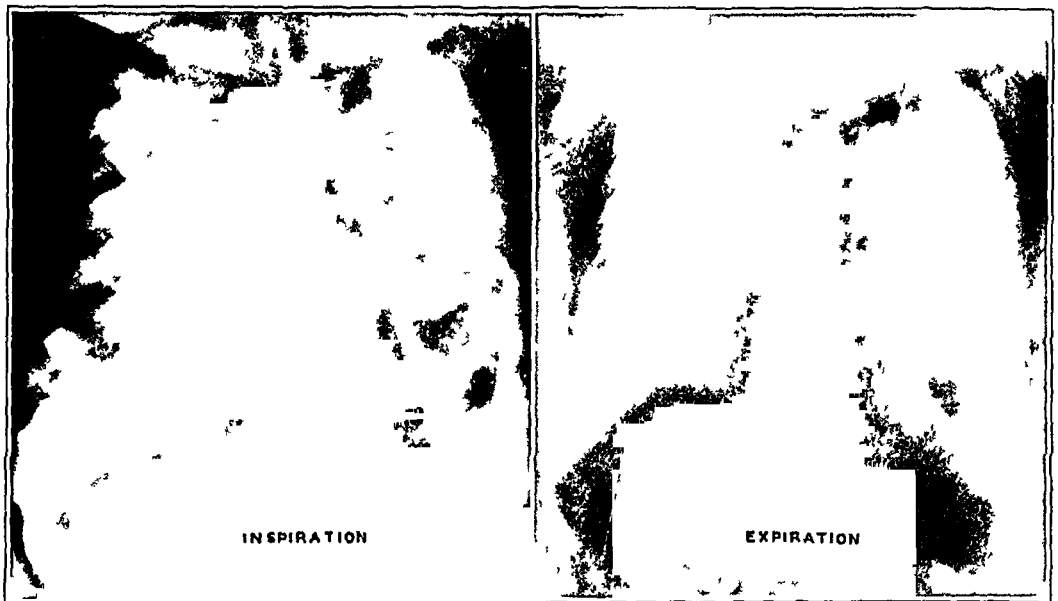


Fig 1—Inspiration and expiration, respectively, showing a definite expansion and contraction of collapsed lower left lobe taking place with each respiratory movement, while the compressed upper part of upper lobe remains unchanged during both respiratory phases. Both inspiratory and then expiratory intrapleural pressures were negative in this case.

expands and contracts to a limited extent with each respiratory phase (Fig 1).

(b) *Static pneumothorax*, or pneumothorax of rest, in which the intrapleural pressure equals that of the intrapulmonary pressure, i. e., both are at atmospheric pressure, and the collapsed lung is in a state of equilibrium or permanent rest (Fig 2).

(c) *Compression pneumothorax*, in which both the inspiratory and expiratory intrapleural pressures exceed that of the intrapulmonary one and the lung is actually compressed, its entire volume being changed and its air capacity reduced to the minimum (Fig 3).

2 Bendove, R. A. The Classification of Artificial Pneumothoraces and Their Corresponding Clinical Value, *Am Rev Tuberc* 10 540 (Jan) 1925.

(d) There exists another type of pneumothorax that cannot be included in the foregoing classification, namely, localized pneumothorax, i e., a small pocket of air in the pleural cavity surrounded and limited by strong pleural adhesions. In such pneumothorax only the immediate lung tissue is compressed slightly, but the rest of the lung keeps on functioning as before, irrespective of the high pressure created in this localized pneumothorax (Fig 4)

Obviously enough, all other factors being equal, the same amount of air introduced in any one of these pleural cavities will occasion different changes in the vital capacity. The smallest variations are noticed in localized pneumothoraces, even such a high positive pressure as $+40$



Fig 2—Inspiration and expiration, respectively, of a case of right pneumothorax in which the inspiratory intrapleural pressure was equal to the intrapulmonary one. There was no expansion of the lobes of this lung during inspiration. The little movement en masse noticed in this lung is due to the motion of the contiguous organs, particularly that of the mediastinum.

$+30$ mercury entails very little reduction in vital capacity, the reason being that all the pressure is exerted only on this localized cavity, whereas the functioning surface of the lung is not encroached on at all. The same principle holds true in a much larger scale in an established pneumothorax of compression, provided the mediastinum is rigid and cannot be displaced. There is no ingress of air into such a compressed lung, and, consequently, it performs no respiratory function. The only volume of air it contains is the "minimal air" enclosed in the collapsed alveoli from which it is never expelled, and if such a condition exists for a long period of time, this small amount of entrapped minimal air becomes absorbed by the blood and the lung will eventually become

entirely solid (Fig 6) It follows that, regardless of the amount of air introduced into such a pleural cavity and the positive pressure created therein, the spirometric readings will be changed very little, as the entire respiratory function is carried out by the noncollapsed lung which, in the presence of a rigid mediastinum, is affected to a very limited extent by the pneumothorax We found that the vital capacity of such cases,

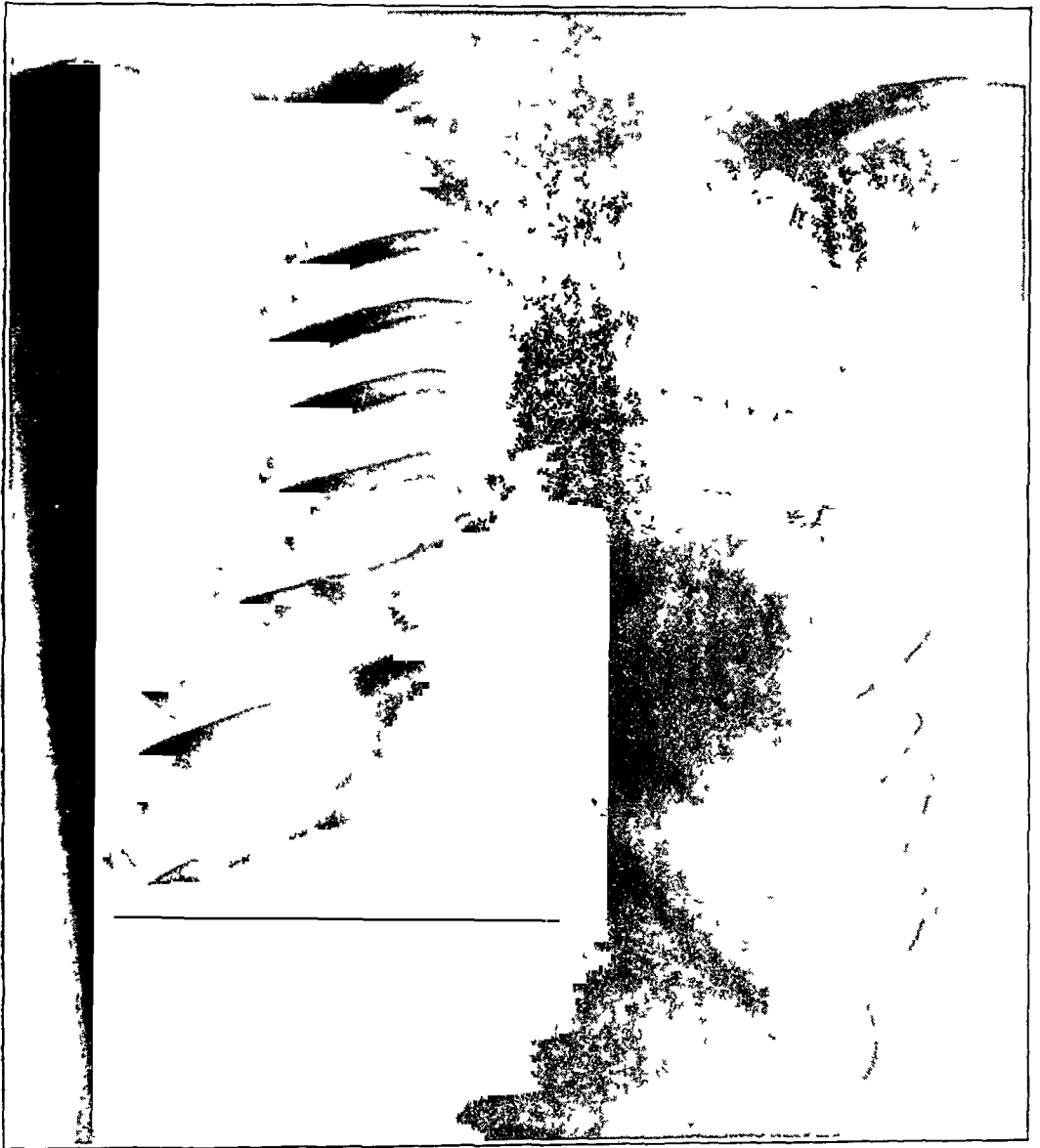


Fig 3—Right pneumothorax, showing lung compressed and reduced to its minimum Both inspiratory and expiratory intrapleural pressures were highly positive

as a rule, bears no relation to the amount of air introduced with each insufflation

Static pneumothorax, or pneumothorax of rest, is a transitory phase which any of the other two types of pneumothorax go through at one time or other In such cases, the vital capacity readings do change with

each insufflation, but these spirometric modifications can be studied more extensively and accurately in expansile pneumothorax, the only type that exhibits a whole series of vital capacity changes during the long course of the collapse therapy. In cases of expansile pneumothorax, each insufflation entailed a reduction in the vital capacity, but



Fig 4—Left localized pneumothorax. A few attempts at induction of pneumothorax resulted in the creation of only two small pockets of air in the first and second interspaces, the strong adhesions preventing the separation of the visceral from the parietal pleura.

never to the extent of the amount of air introduced into the pleural cavity. Furthermore, the same amount of air could at one insufflation bring about a different modification in the vital capacity than at a subsequent one. To explain the phenomenon many factors have to be taken into consideration, and of these the *residual air* is of particular importance.

RESIDUAL AIR OF COLLAPSED LUNG AND ITS RELATION TO VITAL CAPACITY

The vital or the respiratory capacity includes in itself the tidal air, the complemental or the most forced inspiratory air, and the supplemental or the most forced expiratory air. But, even after the entire supplemental air has been driven out, there still remains in the lungs 1 000 c c or more that is never expelled and is designated as the residual air. Whether there exists a constant between the vital capacity and the residual air has not been determined yet, though they seem to be inversely proportional to each other, i e, the greater the residual air

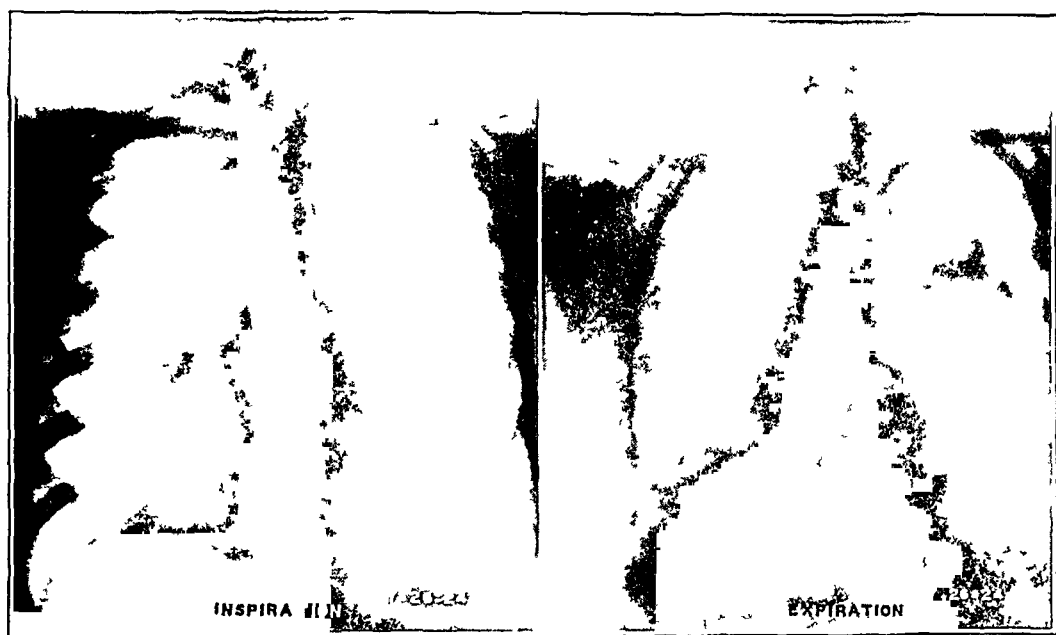


Fig 5—Left pneumothorax, showing lower lobe expanding to its full capacity during inspiration, driving all the air into upper part of pleural cavity around the diseased, inelastic portion of lung, and contracting a great deal during expiration. Two hundred cubic centimeters of air introduced into this pleural cavity brought an increase of 100 c c in the vital capacity. Larger quantities injected never reduced the vital capacity by more than one third of their own amounts.

the smaller the vital capacity, and vice versa. Lundsgaard and Van Slyke³ found that the residual air is increased in early cases of pulmonary tuberculosis, and they are of the opinion that this is the cause of the diminution of the vital capacity of such cases. The small vital capacity of emphysematous cases is also explained on the basis of an excessive residual air of the overinflated and poorly elastic lung. On the other hand, it was found that athletes have a high vital capacity and a very low residual air, because the well developed muscles of the chest and, particularly, those of the abdomen press tightly on the abdominal con-

³ Lundsgaard, C, and Van Slyke, D. R. Studies of Lung Volume, J Exper Med **27** 129, 1918.

tents, which, in turn, push the diaphragm high into the thoracic cavity during expiration and squeeze out most of the air from the basal lobes.

Yet, even after the most forcible expiration, the lungs are far from being entirely collapsed, so long that the thorax remains closed. The reason is very simple: the lung with its contained air, which presses

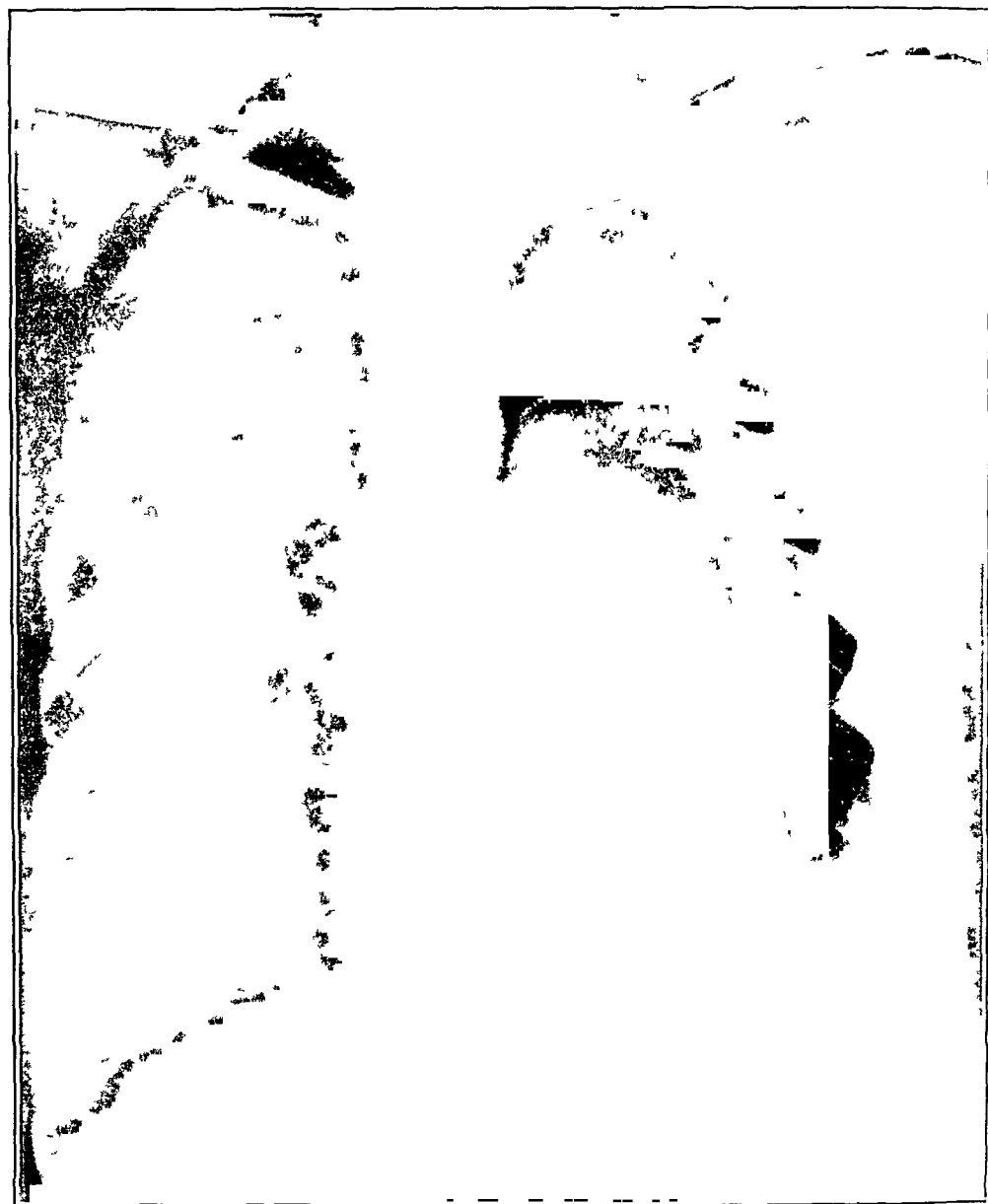


Fig 6—Long standing hydropneumothorax, showing a compressed, almost consolidated left lung, the functional capacity of which is entirely lost. More than 3,000 c.c. was aspirated within a month, but the vital capacity remained as low as before.

against the chest wall, cannot diminish its volume more than the volume of the thoracic cavity, as the expiratory intrapulmonic pressure is always higher than the intrathoracic pressure. Those two antagonistic forces,

i e., the positive intrapulmonary pressure and the negative extrapulmonary, or intrapleural pressure, keep the elastic lung from being collapsed and emptied entirely. When we introduce gas into the pleural cavity, we gradually loosen this negative tie and allow the lung to collapse on itself, so that not only the supplemental air is expelled during expiration but also a part of the residual air is driven out. This expelled residual air, which never participates in the vital capacity of the physiologically functioning lung, becomes an integral part of the vital capacity in expansile pneumothorax and is of clinical importance as it alleviates the dyspnea or prevents it altogether, as will be commented on later. The amount of residual air driven out from the lung stands in direct relation to the rise of the intrathoracic pressure, which is now to be considered.

INSPIRATORY AND EXPIRATORY PRESSURES IN THE INFLATED PLEURAL CAVITY AND THEIR EFFECT ON VITAL CAPACITY

Physiologically, the inspiratory intrathoracic pressure is always more negative than the expiratory pressure, this allows the lung to be much more expanded during inspiration than during expiration. The same holds true in pneumothorax. The expiratory pressure is always greater than the inspiratory pressure, and so long as the latter remains below the intrapulmonary pressure the lung is able to expand with each inspiration, and the more the expansion, the greater is the ingress of air, i e., the greater is the complemental air. When a certain quantity of air is introduced into the pleural cavity, it increases the inspiratory intrapleural pressure and impedes the expansion of the lung during inspiration, which means a curtailment in the complemental air, and thus a decrease in the vital capacity. This reduction of the vital capacity would probably be equal to the amount of air introduced, were it not for the expiratory intrapleural pressure, which also is increased, compressing the lung more effectively during expiration, and driving out not only the supplemental air but also a part of the residual air. The higher the expiratory intrapleural pressure, the greater is the amount of residual air driven out. We thus have created two conditions in the pleural cavity which work in opposite directions, as far as the vital capacity of the collapsed lung is concerned: one, the increased inspiratory intrapleural pressure, tends to curtail the vital capacity, the other, the elevated expiratory pressure, augments it. The amount of reduction of the vital capacity will be equal to the diminution in the complemental air minus the residual air expelled. Both may be affected simultaneously or either one separately, depending on the new pressures created in the pleural cavity. Thus,

(a) If the inspiratory pressure is not reduced very much and the expiratory air is brought slightly above the atmospheric then the vital

capacity will be reduced very little because the complementary air is not reduced much, whereas the positive pressure established in the pleural cavity during expiration exerts itself mostly on the lungs, compressing them and letting the residual air come into play. Case 1 (Fig 7) illustrates it clearly. The vital capacity prior to the insufflation was 3,100 c c, and, after the introduction of 600 c c of air into the pleural cavity, it decreased to 2,900 c c, the actual reduction being only 200 c c though 600 c c was injected. The preinsufflatory intrapleural pressures were -20 -7 , and the postinsufflatory ones were -14 $+4$, i e, the complementary, or inspiratory, air was not reduced very much, and this

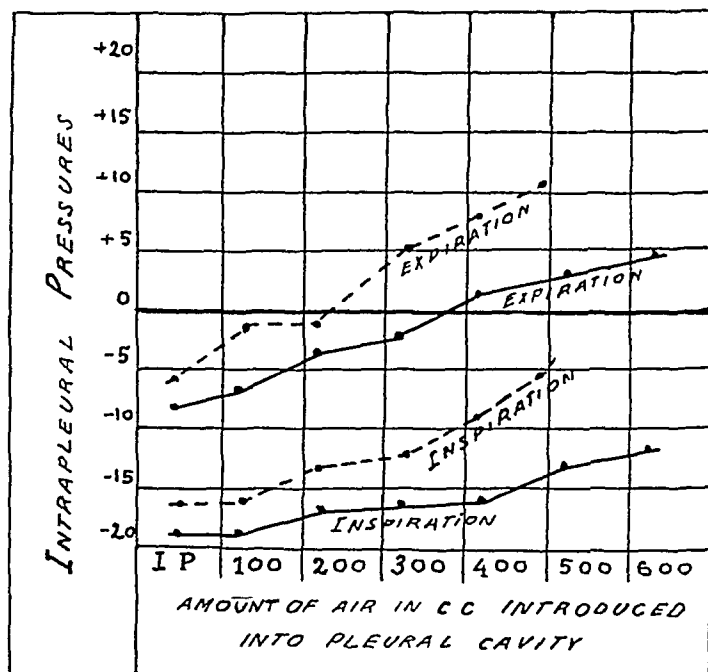


Fig 7—Solid line (Case 1) vital capacity prior to insufflation, 3,100 c c, after insufflation, 2,900 c c, a reduction of only 200 c c, although 600 c c were introduced, broken line (Case 2) preinsufflatory vital capacity, 2,300 c c, postinsufflatory vital capacity, 2,000 c c, a reduction of 300 c c after injection of 400 c c

curtailment was made up for by the residual air, which was forced out of the lung by the positive expiratory pressure

(b) When the inspiratory pressure is raised to about the atmospheric, which means that practically all the complementary air of the lung has been cut out, then, even in the presence of a high expiratory pressure the vital capacity will be reduced a great deal (Fig 7, Case 2). The explanation lies in the fact that even if the entire residual air could have been driven out from the compressed lung during expiration, its amount would always be smaller than the inspiratory complementary air, which has been reduced to a minimum

(c) When the inspiratory pressure is reduced and the expiratory pressure remains more or less stationary, the vital capacity is diminished almost to the amount of air introduced (Fig 8, Case 3) Such cases usually have a labile mediastinum, which becomes displaced toward the untreated side, encroaching on the respiratory function of the contralateral lung (This factor is taken up in detail under a separate heading)

(d) In compression pneumothorax in which the lung takes no part in the respiratory function, the preinsufflatory and postinsufflatory vital capacities are always about the same (Fig 8, Case 4) The difference

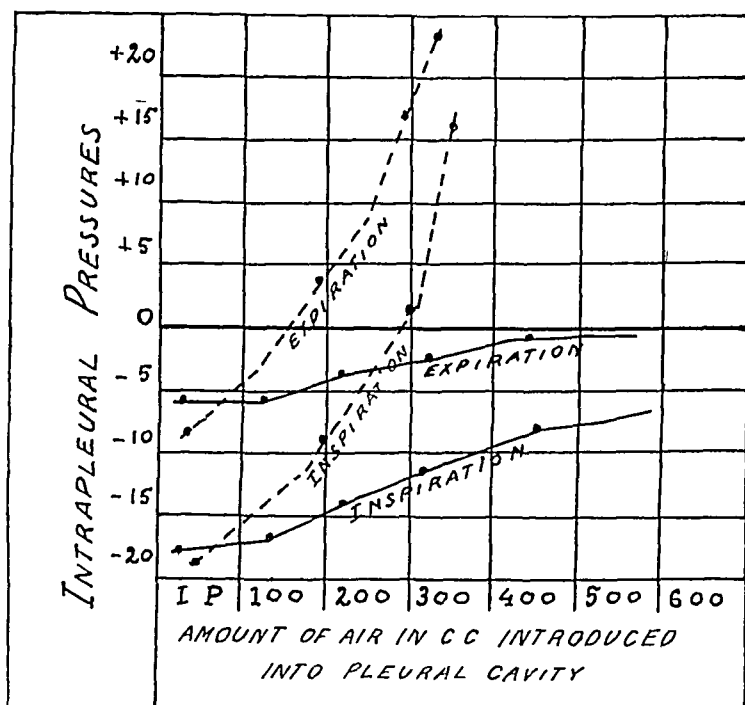


Fig 8—Solid line (Case 3) preinsufflatory vital capacity, 2,800 cc, postinsufflatory vital capacity, 2,400 cc, broken line (Case 4) vital capacity before and after insufflation of 1,500 cc

between the inspiratory and expiratory intrapleural pressures is simply due to the difference between the dimensions of the hemithorax in inspiration and expiration, and since such patients usually possess a rigid mediastinum that offers the same resistance to the introduced air as the chest wall does, a low negative intrapleural pressure is rendered highly positive very rapidly. This cannot happen in expansile pneumothorax when the lung is still functioning and the pressure of the insufflated air exerts itself first on the lung itself. The resistance which the lung offers to the air introduced into the pleural cavity also varies a great deal with the degree of elasticity which its tissues possess at the time of the insufflation.

HOW VITAL CAPACITY IS MODIFIED BY PATHOLOGIC AND
PHYSIOLOGIC STATUS OF COLLAPSED LUNG

The anatomic lung tissue is very elastic and possesses both the expansile and contractile forces to the same degree. Pathologic involvement of the lung may attenuate, injure and destroy entirely either the expansibility alone, or the contractility alone, or both of them simultaneously. In any infiltrative condition of the lung, the expansibility is the first to suffer while the contractility remains more or less intact, hence, the tendency of such tissue to undergo atelectasis. In emphysema, the reverse takes place, the contractility is attenuated whereas the expansibility is intact, leading to an overinflation. When the infiltrated lung tissue undergoes caseation, it loses all its elastic characteristics, both expansile and contractile, and becomes soft and friable. As the tissue goes through all the gamut of destruction and repair to fibrosis it becomes hard and unyielding, but with a strong tendency to contract.

The altered histological architecture of the tuberculous lung treated with artificial pneumothorax exhibits, as a rule all the modified elastic characteristics just enumerated, and consequently it reacts differently to the gas introduced into the pleural cavity. The infiltrated and caseous areas will become compressed at once, the fibrosed areas will yield very slowly but once "caved in" will remain so all the time, whereas the uninvolved portion of the lung will continue to expand and contract with each respiratory movement. So long as the intrapleural pressure is kept lower than the intrapulmonary pressure, the lung will extend to a limited extent with each inspiration, but as soon as the inspiratory intrapleural pressure reaches that of the intrapulmonary one, the expansibility of the lung tissue cannot assert itself any more and the lung remains compressed even during inspiration. If such a high positive intrapleural pressure is maintained for a long time, the expansibility of the lung tissue may become injured and never regain its former physiologic limit. In expansile pneumothorax however we always leave the intrapleural pressure below the atmospheric and the uninvolved portion of the lung continues, therefore, to function within certain confines during the entire course of the treatment.

It should be taken into consideration that the diseased edematous alveoli contain very little air and much less pressure is required to render them entirely airless and close them up completely. Consequently, there is no access of air into such alveoli, and they cease to dilate with each inspiration, it being one of the main desideratum of pneumothorax therapy to keep the diseased alveoli from to and fro motion synchronous with the respiratory movements. As we progress in the course of therapy by collapse of the lung, these atelectatic alveoli become fibrosed and fail to reinflate, even in the most forcible inspiration, they remain permanently compressed and contracted. A most interesting phenomenon

occurs when gas is introduced into such a pleural cavity. The gas accumulates mostly around these diseased areas, no matter where they are situated in the upper or lower lobes, during inspiration, while, in expiration, the gas is distributed evenly from apex to base. Figure 5 exhibits this phenomenon saliently. The explanation offered is that there is no access of air into the diseased, contracted areas of the upper lobe, during inspiration, whereas the lower uninvolved lobe inflates freely with the inspiratory movement and replaces the air in the empty space left by the contracted tissue, during expiration, on the other hand, this functioning part of the lung becomes also collapsed and the air distributes itself evenly throughout the pleural cavity.

What is of greatest interest, as far as spirometry is concerned, in such cases is the fact that a small amount of air introduced into the pleural cavity brings about a slight increase in the vital capacity instead of a decrease. In the case just cited, an introduction of 200 c c entailed, as we anticipated, an increase of about 100 c c in the vital capacity. The explanation is to be sought in the mechanodynamic forces created in this hemothorax, during inspiration, all the dimensions of the thorax increase and the uninvolved elastic part of the lung expands freely, driving all the air into the vacant area left by the nonfunctioning, contracted portion of the lung, whereas, during expiration, the functioning portion recoils on itself, and all the diameters of the thorax decrease and exert an equal pressure throughout, so that the air becomes evenly distributed and forces the residual air from this functioning portion of the lung. If, however, the amount of air insufflated is being increased, it begins to encroach also on the inspiratory expansion of the functioning portion of the lung, cutting short its complementary air. This impediment of expansion stands in direct relation to the amount of air introduced and the inspiratory intrapleural pressure created. Should a high intrapleural pressure be maintained for a long period of time, keeping the uninvolved part of the lung in compression, its expansile force becomes inhibited or greatly injured, and, of course, a reduction of the vital capacity ensues. That is why it is much better to give smaller amounts with lower intrapleural pressure, repeated quite frequently, than to give large amounts with a high intrapleural pressure.

Once the expansibility of the lung tissue has been inhibited or injured, it can never be regained. The contractile force preponderates and its tendency to atelectasis is augmented. Thus, we have patients treated with artificial pneumothorax under high intrapleural pressure, their lungs, having been kept compressed for months, were allowed to expand gradually, by reducing the insufflatory amounts and the intrapleural pressure. Such patients gain gradually in their vital capacity, but every insufflation curtails their vital capacity much more than the amount of air introduced into the pleural cavity, because of the marked

tendency of this lung tissue to undergo atelectasis. But, after a few days, particularly if they are made to breathe deeply, they regain the former amount of vital capacity. This shows that their expansibility has not been permanently injured, but has been greatly attenuated by the long standing compressed condition. However, those lungs which have been kept compressed for many years fail to re-expand, and the vital capacity of the patients is at a low ebb. Fluid causes even a greater injury to the expansibility of the lung tissue and, if not removed in time may occasion a permanent compression of the lung and a complete reduction of its respiratory capacity (Fig 6)

TEMPORARY AND PERMANENT EFFECT OF PLEURAL EFFUSION ON VITAL CAPACITY

As already intimated, the extent, character and permanence of the loss of the expansile quality of a compressed lung stands in direct relation to the nature and the duration of the underlying cause, hence the longer the fluid is left in the pleural cavity, the greater injury will it inflict on the expansibility of the lung tissue and, therefore, also on the vital capacity. The chart (Fig 9 Curves 1 and 2) of cases of hydropneumothorax illustrates lucidly that not only the fluid, but also, to a greater extent, its duration in the pleural cavity causes a marked crippling of the respiratory capacity of the lung. Curve 1 represents a case of pleural effusion of more than a year's duration. Before aspiration, the vital capacity of the patient (a man) was 2,200 c c, and the withdrawal of 1,800 c c of thick, yellow fluid replaced by 800 c c of air, brought no change in the vital capacity. Daily spirometric readings for four consecutive weeks disclosed no essential changes (the small changes noticed on the chart are due to the physiologic variations observed in any serial spirometry). At the end of three weeks, the pleura was insufflated with 300 c c of air, it gave rise to a high intrapleural pressure, but no alteration in the vital capacity took place.

Curve 2 exemplifies the effect of the timely removal of fluid from the pleural cavity on the rapid recuperation of the respiratory capacity of the lung. This was a case of hydropneumothorax of about two months' duration. The vital capacity of the patient (a man), before aspiration, was 2,000 c c. After the withdrawal of 1,400 c c of clear fluid and its replacement by 800 c c of air, the vital capacity increased to 2,200 c c. Daily spirometric readings showed a gradual but persistent increment of the vital capacity, so that at the end of three weeks it reached 2,800, i e., an increase of 800 c c, almost half the amount of his vital capacity prior to the aspiration. When 500 c c of gas were now introduced into the pleural capacity, it occasioned a reduction in the vital capacity to the same amount. This seldom happens in other cases of expansile pneumothorax. However, in four days, the vital capacity returned to what

it was before the induction of gas. The vital capacity modifications in the subsequent refills did not differ in any way from those described under expansile pneumothorax.

In Case 1, the compressed lung, under the fluid for more than a year, became completely atelectatic and probably fibrosed, lost its elasticity and could not expand any more, even after the removal of the primary cause. In Case 2, the elasticity of the lung did suffer slightly under the pressure of the fluid, but, as soon as the cause was removed, it took a comparatively short time for the atelectatic lung to regain its expansibility, gradually but persistently, the vital capacity keeping pace with it.

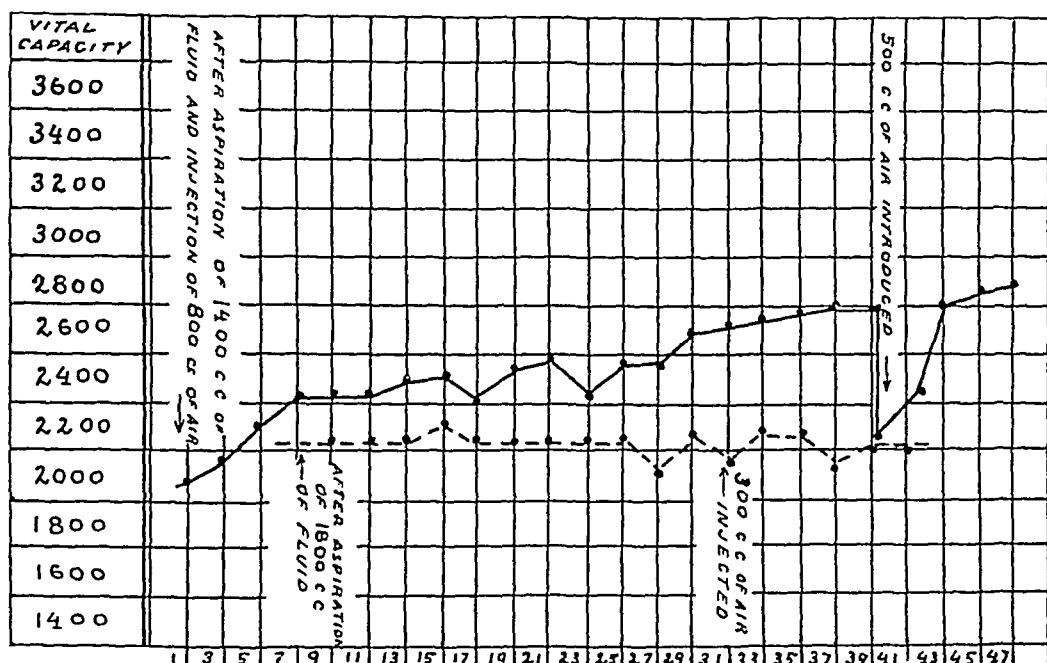


Fig 9—Broken line case of left pleural effusion of about one year's duration, showing no change in the vital capacity after aspiration, solid line case of right hydropneumothorax of about two months' duration, showing a gradual increase in the vital capacity after aspiration.

This is the best argument in favor of aspirating pleural fluid as soon as possible and replacing it by air, which is much easier to manage, exerts less compression on the lung, and is absorbed much more quickly.

Furthermore, fluid always gravitates to the bottom, compressing the basal lobe, no matter whether it is diseased or not, whereas air distributes itself according to the existing pneumodynamics, which makes it accumulate mostly around the diseased areas.

It is worthy of notice that, even when we replaced the aspirated fluid with the same amount of air, the vital capacity was invariably greater than before aspiration. This might be attributed to the greater compressibility of air than fluid and to the fact that air can be made to occupy less space during inspiration, allowing the lung to expand and breathe in more air with each inspiratory phase.

MOVEMENTS OF DIAPHRAGM AND MEDIASTINUM WITH REGARD TO
VITAL CAPACITY OF COLLAPSED AND CONTRALATERAL LUNGS

The relation of the movement of the diaphragm to the vital capacity will be reported in a separate contribution on phrenodynamics of which we are now making a special study. Suffice it to mention here that when the diaphragmatic function is not injured it assists a great deal in increasing the vital capacity by compressing the lower lobe during expiration, this is particularly true when the basal lobe is the only functioning one. In case of paralysis of the diaphragm, a part of the air injected into the pleural cavity is wasted by keeping it down at a lower level than its physiologic one, and it should be taken into consideration by the operator when insufflations are made.

The rôle of the mediastinum in artificial pneumothorax, has been discussed fully in my work on the classification of artificial pneumothoraces and their corresponding clinical value² to which the reader is referred. Its mobility has little bearing on the vital capacity of the collapsed lung, but it is one of the chief factors in modifying the vital capacity of the untreated lung. If it is very rigid and stable it never becomes deflected into the contralateral side, no matter how high the intrapleural pressure is raised and consequently, no encroachment is made on the respiratory surface of the other lung. But if the mediastinum is labile, it becomes displaced into the other hemithorax, the higher the intrapleural pressure, the greater is the displacement and the more is the embarrassment of the contralateral lung. In the presence of a labile mediastinum, no compression pneumothorax can be obtained without causing marked respiratory distress and dyspnea, not only because of the marked reduction of the vital capacity of the treated lung, but also on account of the compression of the other lung, caused by the displaced mediastinum and diminishing its respiratory capacity.

We had a case of a right spontaneous pneumothorax in which the mediastinum was thrust beyond the nipple line of the other hemithorax (Fig 10). The patient was very orthopneic and his vital capacity was, at that time, only 800 c c. Only after the withdrawal of 1,000 c c of air from the pleural cavity could he lie on his back without gasping for air, though the intrapleural pressure still remained slightly positive, and the pneumothorax lung was entirely compressed. His vital capacity was then 1,200 c c. Fluoroscopically we observed that the collapsed lung did not function yet, but that the mediastinum was moved more toward the median line, proving beyond doubt that the 400 c c increase of the vital capacity came from the contralateral lung, which was now relieved from the pressure of the previously displaced mediastinum.

Fortunately, most of the patients treated with artificial pneumothorax that require a high intrapleural pressure because of unyielding cavities

abundance of fibrosis and pleural adhesion, have, as a rule, a rigid mediastinum, and therefore a compression pneumothorax can be obtained without encroaching on the respiratory capacity of the contralateral lung. In expansile pneumothorax, in which the intrapleural pressure is always negative, there is very little deviation of the mediastinum to the other side, and the reduction of the vital capacity of the noncollapsed lung is

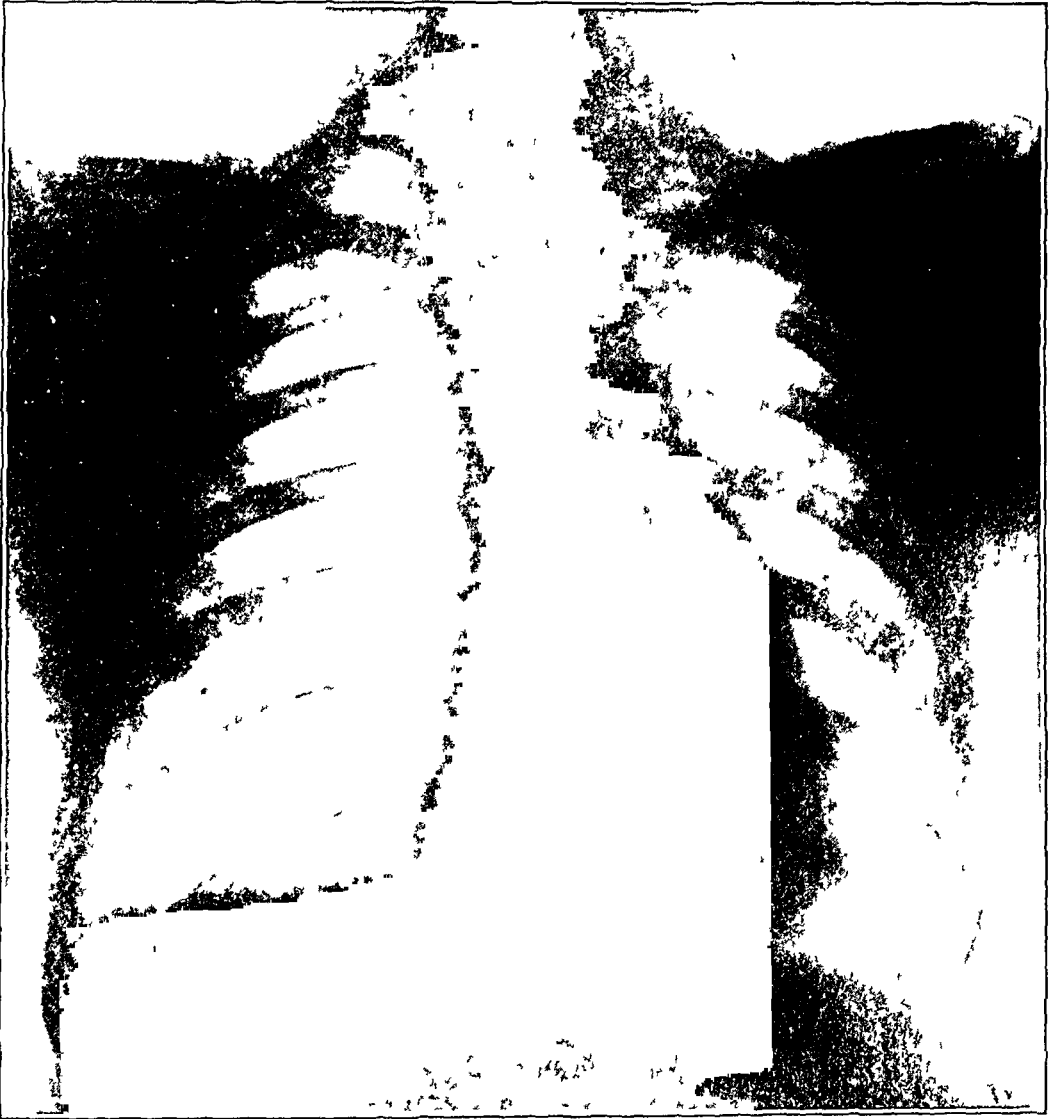


Fig 10—Right spontaneous pneumothorax with considerable displacement of the mediastinum to the left side. The vital capacity, at this time, was 800 c c. After aspiration of 1,000 c c of air from the pleural cavity, the inspiratory intrapleural pressure still remained slightly positive, but the mediastinum was moved toward the median line and the vital capacity increased to 1,200 c c.

negligible. The relations of the amount of air introduced with each insufflation to the amount of air curtailed of the vital capacity will then depend only on the treated lung, and will show all the modifications of the vital capacity, as explained before. However, when the expiratory intrapleural pressure reaches about zero, in the presence of a very labile

mediastinum, then the vital capacity will be diminished to the same amount of air introduced into the pleural cavity, because the other lung also is then being encroached on by the displaced mediastinum

Though the mediastinum is one of the main factors affecting the vital capacity of the contralateral lung, the compensatory ability of the heart and the development of vicarious emphysema are the determining factors in this respect

INFLUENCE OF HEMODYNAMICS ON VITAL CAPACITY OF COLLAPSED AND NONCOLLAPSED LUNG

The interdependence of the circulatory and the respiratory systems is so intimate that any change occurring in one of them is invariably followed by certain changes in the other one. Thus, in case of heart failure, the lung is first to suffer from a venous stasis, and, when an obstruction occurs in the pulmonary circulation, the heart is first to respond to it by a dilatation or hypertrophy of its right ventricle

The resistance of pulmonary circulation is governed a great deal by the degree of lung expansion, both hyperdistention and compression of the alveoli interfere with the circulation of the blood in the pulmonary capillaries. Hypertrophic emphysema furnishes a good illustration of impaired pulmonary circulation because of overdistention of the alveoli the walls of which become thinned out pressing on the capillaries and impeding the blood flow therein, which impediment leads to a dilatation and hypertrophy of the right side of the heart. As an example of interference with the pulmonary circulation because of compression of the alveoli could be cited pleural effusion or pneumothorax

The degree of interference with the circulation in the collapsed lung runs parallel with the amount of compression to which the alveoli are subjected, and accordingly the pulmonary circulation is disturbed much more in compression pneumothorax than in expansile pneumothorax. So long as the blood vessels of the collapsed lung are still patent the blood continues to flow through them, though their tension is very high, but when they become entirely obliterated, the blood stream must, of course, be diverted to other channels or blood vessels. But, in order not to disturb the equilibrium maintained between the systemic and pulmonary circulations the heart has to increase its action and propel the same amount of blood in the same unit of time through the reduced capillary surface. If the reserve power of the heart is not damaged, it compensates well and increases its action, which leads to a dilatation and hypertrophy of the right ventricle or the entire heart, but if the cardiac muscles have been damaged by the long and persistent tuberculous toxemia it fails to compensate and stasis takes place in the pulmonary circulation

Underhill,⁴ in experimenting with ligating one of the pulmonary vessels in animals, found an increase in the blood pressure of the carotid, and he interprets this rise in the systemic blood pressure as a compensation of the heart because of deficient oxygenation. But, if the animal was fatigued and exhausted, a fall in the carotid blood pressure was noticed on ligation of the pulmonary vessels, which meant that the heart was unequal to the strain put on it, and could not send out the same quantity of blood in a given time through one lung only.

The same phenomena we noticed in our patients treated with artificial pneumothorax, some of them showed an increase in the systemic blood pressure after deflation of one lung, and some of them showed a decrease in blood pressure. Those who showed a higher blood pressure after the induction of pneumothorax exhibited also an enlargement of the diameters of the heart, seen distinctly on the roentgenograms. Such patients, as a rule, had only a slight diminution in their vital capacity and, even if the decrease was marked after the first few insufflations, it recuperated gradually and persistently. But those patients who showed a lower blood pressure after pneumothorax exhibited either no change in the size of the heart or it became even smaller, and their vital capacity fell greatly after first insufflations and seldom returned to its former volume.

Rubow⁵ found a decrease of the vital capacity in all cardiac cases, and one of the main causes, he thinks, is that the lung tissue is rendered rigid and inelastic by the capillary vascular turgescence, and dilatation and contraction of the lung are thus interfered with. Another reason given is the protrusion of the distended capillaries into the alveoli which decreases their volume of air. The same is probably true of the lung tissue and the capillaries, in cases treated with artificial pneumothorax for the first few days. But, if the heart compensates well and a vicarious emphysema develops, not only in the contralateral lung but in the functioning portion of the collapsed lung as well, the respiratory capacity reestablishes gradually, whereas, if the exhausted heart fails to compensate against the increased resistance in the pulmonary circulation, a stasis ensues, and a marked fall in the vital capacity remains permanent.

It is obvious that the degree of compensation demanded of the heart is directly proportional to the amount of compression of the lung, hence in those patients whose hearts become anemic and exhausted by the chronic tuberculous toxemia, the deflation of the lung should be done very gradually and with small amounts. Periodic spirometry is of great help in such a case, if the vital capacity falls too rapidly after the first

4 Underhill, S. W. An Investigation into the Circulation of the Lungs, *Brit. M. J.* **11** 779 (Nov. 12) 1921.

5 Rubow, V. In *Kardiale Dyspnea*, *Ergebn. d. inn. Med. u. Kinderh.* **111** 92, 1909.

insufflations, the quantity of gas introduced with the subsequent refills should be diminished, otherwise, marked dyspnea may ensue

WHAT IS THE RELATION OF THE ALTERED VITAL CAPACITY
AND THE MODIFIED FUNCTION OF THE LUNGS
TO DYSPNEA?

A clear distinction between hyperpnea and dyspnea should always be borne in mind. As long as the lungs respond easily and without undue exertion to any urgency for an augmented ventilation, there is merely a hyperpnea, but if the lungs fail to cope with the increased demand for an excessive ventilation, then dyspnea occurs.

Whenever the tidal air becomes insufficient to satisfy the requirements for oxygenation, the complemental air is called on by increasing the depth of inspiration. Peabody⁶ attributes the dyspnea of patients with weak or damaged hearts to their inability to increase their inspiration whenever necessary. The same applies to the dyspnea provoked by any diminution of the respiratory surface due to diseases of the pulmonary parenchyma, the degrees of which are determined chiefly by the extent to which pulmonary ventilation is crippled and the depth of the inspiration inhibited. One would therefore expect a marked dyspnea in artificial pneumothorax cases, in which the respiratory surface is always diminished a great deal. Such, however, was not the case. We were amazed to notice that tuberculous patients with a vital capacity of 1,200 c.c. were very dyspneic and could hardly take a few steps without stopping "for air," whereas pneumothorax patients whose vital capacity was the same or even lower were fairly comfortable, and were up and about without showing any signs of respiratory distress. Paradoxical as it may seem, this is probably due to the greater respiratory efficiency of the expansile pneumothorax lung.

Howell⁷ states that the new-born child has practically no reserve supply of air in the lungs, at each expiration, the lungs are entirely emptied (except for the minimal air). The ventilation of the lung alveoli is correspondingly more perfect than in older persons. The smaller the residual air, the better is the ventilation of the lungs. It follows, therefore, that in moderately collapsed, not compressed lungs, i.e., in a pneumothorax of the expansile type, the ventilation is better than in physiologically functioning lungs, since, as was indicated previously, the residual air is greatly diminished and all the air coming in with the inspiratory movement goes out with each expiration (except the minimal air) and is entirely renewed. This quantity of air, even if it amounts only to 200 or 300 c.c., is of great value in preventing dyspnea,

6 Peabody, F. W., and Wenworth, J. A. *Clinical Studies of the Respiration*, Arch. Int. Med. 20: 442 (Sept.) 1917.

7 Howell, W. H. *Textbook of Physiology*, Philadelphia, W. B. Saunders Company, 1918, p. 654.

because the physiologic tidal air, i e, the new air breathed in at each ordinary inspiration amounts to only 500 c c, but, as the "dead space" occupies about 150 c c, it follows that the ventilation of the lung in normal respiration is only 350 c c. At each inspiration, therefore, only 350 c c of air penetrates into the alveoli, and, since the reserve supply of the lung may amount to 2,600 c c (supplemental air 1,600 c c and residual air 1,000 c c), it follows that the "ventilation volume" of the alveoli is only $\frac{350}{2,600}$, or about one-seventh. But, in expansile pneumothorax, in which all the surface is reduced and the reserve supply is diminished, the "ventilation volume" of each alveolus is much more effective. Another reason for a better ventilation in the expansile pneumothorax lung is the greater volume of blood that runs during the same unit of time through the collapsed lung, i e, the same amount of blood flows through a reduced area, as was discussed previously. This acceleration of blood flow through the pulmonary capillaries brings about a more rapid absorption of carbon dioxide from the alveoli, which in turn provokes a more rapid diffusion of the new air breathed in at each inspiration and, hence a more effective oxygenation.

Of course, in cases of completely compressed lungs, there can be no question of tidal air and reserve air, because such lungs take almost no part in the process of ventilation. Patients treated with compression pneumothorax become very dyspneic and remain so for a long time, unless the other lung compensates well and adapts itself to its new task of performing an almost doubled respiratory function. This vicarious adjustment of the contralateral lung commensurates with the compensatory capacity of the heart, particularly of the right ventricle, which has to propel the blood against an increased resistance, if the cardiac muscle has been damaged by the long continued tuberculous toxemia and its reserve power has been weakened, venous stasis of the lung usually ensues, which entails poor oxygenation and consequently is attended by greater dyspnea.

The relation of dyspnea to the diminution of the vital capacity in expansile pneumothorax and in compression pneumothorax is illustrated in the following two cases.

One patient was given pneumothorax because of a protracted febrile course, unaffected by sanatorium treatment, and the other one, on account of frequently repeated and copious hemoptysis. The first patient was given small amounts of gas, and his intrapleural pressure was kept negative all the time, the vital capacity was reduced 10 per cent the first day, and it was never decreased to more than four-fifths of what it was before the initiation of pneumothorax. He was dyspneic only the first few hours after induction of pneumothorax, but the next day came to the operating room without exertion, and none of the subsequent reinsufflations were attended by dyspnea. The other case was given large amounts of gas and a positive intrapleural pressure was created in order to afford an effective ligation of the bleeding blood vessels, his vital capacity was reduced about 45 per cent, he could hardly take a few steps without support, and remained dyspneic for a long period of time.

CLINICAL AND PROGNOSTIC SIGNIFICANCE OF PERIODIC
SPIROMETRY IN PNEUMOTHORAX THERAPY

A variable series of vital capacity changes are observed, during the long course of artificial pneumothorax, in accordance with the manifold pathologic and functional modifications that necessarily take place throughout the evolution of the collapse therapy. These spirometric readings, if studied carefully and interpreted properly in conjunction with the physical signs and roentgenologic findings, afford a great deal of information as to the status of the collapsed and contralateral lungs, particularly of their respiratory capacity and functional recuperation. Indeed, whereas the symptomatology is indicative more or less of the activity of the lesion, and the roentgenography betrays the anatomic-pathologic changes of the lung, spirometry is the only means by which we measure the recuperation and compensation of the physiologic function of the lung, and is of great help in prognosticating the success or failure of the therapy.

After the initial injection of gas into the pleural cavity, there is always a fall in the vital capacity corresponding to or exceeding the amount of air introduced, this being due not only to the actual reduction of the respiratory surface, but also to the altered intrathoracic pressures to which the circulatory and the respiratory systems are trying to adjust themselves, bringing the hemodynamics and the pneumodynamics to a new equilibrium. The decreased ventilation of the lungs and the deoxygenation is made up for by the tachypnea which is marked the first few hours after the initiation of pneumothorax. The rate of breathing, however, soon resumes its former limit and the vital capacity increases slightly though it still remains below what it was prior to the initiation of the treatment. But, if the initial pneumothorax entails a decrease of more than 15 per cent of the respiratory capacity, dyspnea ensues, and the patient feels very uncomfortable. The initiatory amount of gas introduced into the pleural cavity should therefore never be greater than one-sixth of the vital capacity of the patient, the smaller the quantity the less the disturbance in the respiratory and circulatory system. If the vital capacity, for instance, is 3,000 c c, about 400 c c of air is to be given at the initial pneumothorax, if the vital capacity is only 2,000 c c, not more than 300 c c of air is advisable for the first injection. The few, immediate subsequent insufflations should also be commensurate, in the same way, with the vital capacity of the patient.

In rare cases, the vital capacity increases after induction of pneumothorax, if the amount of gas injected was small. These are patients who had severe pleuritic pains, inhibiting any attempt at deep breathing, but, now that a thin layer of air separates the visceral from the parietal pleura the deepest inspiratory movement can be accomplished, provided the

heart is in good condition and there is no great alteration in the intrathoracic equilibrium. In other cases, there occurs no change at all in the vital capacity after induction of pneumothorax. These are patients with marked pleural synechiae, leading to the formation of a small localized pneumothorax, leaving the rest of the lung unaffected. The vital capacity of such cases shows no further modification incidental to the pneumothorax, and, as a rule, these cases benefit very little by the treatment.

The immediate reinsufflations, which are spaced at intervals of two or three days, cause a further diminution of the vital capacity, which is very gradual and persistent. Should a sudden drop occur in the vital capacity during this time, a careful examination of all the factors affecting it is to be made, physically and roentgenoscopically, and the subsequent refills will be modified accordingly. A spontaneous pneumothorax might develop and, with the exception of a transient dyspnea, cause no symptoms at all if a rigid mediastinum that holds off the pressure from the contralateral lung exists, and the heart has, by this time, compensated sufficiently to be able to propel the blood against the increased resistance. But the vital capacity is greatly reduced, and dyspnea, on exertion, is inevitable. No refills should be made into such a pneumothoracic cavity, as it may lead to a complete compression of the lung and consequently to a permanent decrease of the respiratory capacity, but the patient should be made to blow the spirometer every day in order to achieve an expansion of the nondiseased alveoli and return to the former vital capacity. If the spontaneous pneumothorax is distressing, air should be aspirated from the pleural cavity until negative intrathoracic pressure is reached, which allows the lung to dilate with each inspiratory movement, and, of course, the vital capacity is at once elevated.

When no complications occur and the deflation of the lung is graduated slowly and systematically, then the functional modifications of the respiratory capacity are very characteristic, as studied by the "preinsufflatory" and "postinsufflatory" spirometric readings.

A steady step by step decline of the vital capacity is noticed with each insufflation, the cumulative effect of which results in a marked curtailment of the respiratory capacity, but with no distress to the patient, who has little by little adjusted himself to this gradually modified ventilation of the lung. However, a limit is reached which may be looked on as the turning point in the evolution of the collapse therapy when the vital capacity begins to ascend gradually or remains stationary for a time and then increases very slowly but persistently. This transitory point of the vital capacity, in the long course of artificial pneumothorax, is of great value in prognosticating the ultimate result of the therapy, provided no complications set in. It varies with each case in time and amount, it may take place at any time during the course of the treatment and at

any level below the physiologic vital capacity for that person. If it occurs between the first and fourth month after the initiation of the treatment and if the vital capacity at that period is not below 70 per cent of the physiologic vital capacity for that person, the prognosis is very good. Even when the vital capacity is below 70 per cent but remains stationary or increases gradually without marked fluctuations, the prognosis is also favorable.

A stationary vital capacity, regardless of whether it is high or low, always signifies that the lesion became arrested or quiescent. In a large group of cases not treated with artificial pneumothorax, we found that the same lesion, when it became active, was liable to reduce the vital capacity to about half its original amount, and therefore those who had a labile lesion exhibited marked fluctuations in their serial spirometric measurements, whereas those who had a quiescent lesion showed either an increasing or a persistently stationary vital capacity. Marked fluctuations in the vital capacity always meant an active lesion. The same holds true in the cases treated with artificial pneumothorax. When the vital capacity becomes stationary or begins to increase very gradually but persistently, it indicates that the diseased areas have become compressed and quiescent, ceasing to discharge toxins into the system, that a complete equilibrium has been established in the respiratory system, and that not only the contralateral lung has developed compensatory emphysema, but also that the functioning portion of the homolateral lung is adjusting itself to its modified way of functioning and is able to develop a vicarious emphysema to expand and occupy the spaces of the hemithorax left empty by the contracted diseased parts of the lung. It is this gradual but steady enlargement of the expansion that brings about the slowly progressive increase in the respiratory capacity, provided no complications arise.

Two phenomena are thus taking place simultaneously as far as the treated lung is concerned. The diseased portion contracts and shrinks continually, while the uninvolved portion keeps on dilating progressively. This should be borne clearly in mind whenever reinsufflations are made. These inflations are not given with the purpose of "knocking the entire lung out of commission," but to assist in keeping the diseased parts in their compressed and contracted position, allowing at the same time the uninvolved portions of the lung to expand and contract more or less freely with each respiratory movement. The amount of air introduced now into the pleural cavity should never curtail more than half its own quantity of the vital capacity, and as we progress successfully in the course of the treatment, the curtailment should be diminished correspondingly, and the introduced volume of air should bring about a reduction in the vital capacity amounting to less than one-fourth its volume. The reasons have been discussed fully in the previous chapters.

Suffice it to mention here again that, all the other factors being equal, it depends wholly on the degree of expansion of the uninvolved portion of the lung, the greater the expansion, the more air is breathed during inspiration, and the more air can be expelled during expiration, consequently, the greater increase in the vital capacity. Of course, this holds true only when moderate amounts of air are administered. If, however, a large quantity of air is introduced, it encroaches also on the functioning tissue, and naturally a great reduction in the vital capacity ensues. It is therefore advisable to inject always about the same amount at the same intervals of time, and note the difference between the preinsufflatory and the postinsufflatory vital capacity, which should become smaller as we advance in the treatment. Each case should be studied roentgenoscopically before and after every inflation, the expansile capacity of the undiseased portion of the lung observed closely, frequent physical examinations of the chest made, all these findings corroborated with the serial spirometric readings, and the insufflations modified accordingly.

If fluid develops, the drop in the vital capacity may be very marked and sudden, both because of an actual reduction of the respiratory capacity and because of pyrexia, probable toxemia and increased strain on the heart preventing the taking of a deep breath. Fluid should never be left in the chest for a long time, as it is liable to cause a permanent injury to the expansion of the lung tissue. It should be aspirated as soon as possible and replaced partially by air, which is managed much more easily, is absorbed more quickly, and allows the undiseased tissue to expand. If not much injury has been done to the elastic quality of the tissue, it will show itself immediately after the aspiration of the fluid by an increase in the vital capacity. The volume of air replaced should always be less than the amount of fluid aspirated. The patient should blow the spirometer every day from three to five times, the gradual increase in the vital capacity should be noted, then treated as a usual case of expansile pneumothorax.

No untoward results come from frequent blowing of the spirometer, even during the first few weeks of the treatment, though, as a rule, we do not let patients blow more often than before and after each insufflation. But as soon as the patient becomes more or less symptomless, expectoration is brought to a minimum and all signs indicate that the lesion has become quiescent, we let the patient take the vital capacity very frequently, first, in order to have a serial record and, second, to enable the expansile portion to dilate more and more. This displaces the gas into spaces left by the compressed, diseased parts of the lung and thus compresses them more. The expansile phenomenon is displayed in such cases most vividly. If not interfered with by improper treatment and complications, these functioning lobes reexpand finally to their full capacity, and, owing to the compensatory emphysema developed in them,

their functional recuperation is very marked. The vital capacity of such cases is greater than what it was prior to the initiation of pneumothorax, and, in two of our cases, the vital capacity came up to more than a 100 per cent of the calculated physiologic vital capacity for these patients.

It should be emphasized again that all serial spirometric changes described above accompany only cases treated with expansile pneumothorax, but if compression pneumothorax is instituted and maintained, no recuperation of the functional capacity can be expected. We seldom treat with compression pneumothorax now, only, in case of severe hemoptysis, we create a highly positive intrapleural pressure in order to effect a better ligation of the bleeding blood vessels, but even in such cases we soon allow the expansibility of the undiseased portion to assert itself. Of course, when fibrosis preponderates all over the lung, no reexpansion can be expected, and, once compressed, the lung will remain so forever, requiring refills every now and then to prevent deformity of the chest. The vital capacity of such patients is always at a very low level and can never be increased. This might be used as the strongest argument in favor of initiating pneumothorax in early cases and not waiting till the patient and the lung have lost their recuperative ability. At that time, pneumothorax can offer only ameliorative results, whereas, if it is administered to patients in the first or in the second stage of pulmonary tuberculosis, complete anatomic and functional recovery can be expected.

In a later contribution on the results of artificial pneumothorax, there will be discussed fully the relation of age, sex, side of pneumothorax, and involvement of the contralateral lung to the anatomic and particularly to the functional recovery. Suffice it to mention here that we found that the recuperation of the functional ability of the lung and return to their physiologic vital capacity was more frequent in young persons than in old ones, owing, no doubt, to the better reserve power of their hearts and the more pronounced elastic quality of their lung tissues, enabling the development of a marked compensatory emphysema.

The vital capacity percentage of the subject before the initiation of the pneumothorax also has a great bearing on the ultimate result of the treatment. Those patients whose vital capacity is not much below their calculated physiologic normal do, as a rule, much better than those who have a low vital capacity, and if the expansile pneumothorax is managed properly and graduated systematically, the pulmonary function recuperates slowly but persistently, and the lungs may eventually attain their complete physiologic respiratory capacity.

SUMMARY AND CONCLUSIONS

- 1 The vital capacity modifications during the course of artificial pneumothorax treatment are very characteristic, and careful periodic

spirometry can therefore afford a great deal of information about the respiratory recuperation of the lungs and how to regulate the insufflatory amounts, and can be used as an additional criterion in prognosticating the success or failure of the therapy

2 There is no quantitative correspondence between the amount of air introduced into the pleural cavity and the reduction of the vital capacity, but the same quantity of air at various inflations brings about different results depending on the status of the other factors modifying the vital capacity in pneumothorax, the most important of which are

(a) The residual air of the collapsed lung which, owing to the increased expiratory intrapleural pressure created in the treated hemithorax, is expelled with each deep expiration and thus made to participate in the vital capacity

(b) The newly established intrapleural pressures which antagonize each other as far as the vital capacity is concerned a high inspiratory pressure entails a diminution in the complementary air, thus reducing the vital capacity, whereas a high expiratory pressure augments the supplemental air, which means an increase in the vital capacity

(c) The extent of the pathologic involvement and the functional ability of the treated lung which depends on the elastic quality of the tissue, the greater the injury to the *expansibility* of its elastic tissue, the greater and more permanent is the decrease in the vital capacity

(d) The mediastinum which, if rigid, has little effect on the vital capacity, but, if labile, encroaches on the contralateral lung when a high intrapleural pressure exists and causes a diminution in the vital capacity

(e) The heart, when weakened by the continuous tuberculous toxemia, may fail to meet the demand for additional work in propelling the same amount of blood against an increased resistance in the pulmonary circulation, venous stasis and turgescence of the lung blood vessels follow, leading to a diminution in the vital capacity The demand on the reserve power of the heart stands in direct relation with the degree of deflation of the lung

3 Patients with a low capacity who are treated with pneumothorax have much less dyspnea than other tuberculous patients having the same low vital capacity, this being due to the residual air of the treated lung which is also breathed out and renewed with each increased respiratory movement, rendering the "ventilation volume" of each alveolus much more effective If, however, the introduced air into the pleural cavity entails more than one fifth of the vital capacity of the patient, dyspnea ensues

4 There are three distinct phases in the vital capacity during the course of the treatment a gradual decrease, a stationary period, and slowly progressive increase If the initial decrease is sudden and marked,

in spite of the small amount of air injected at each insufflation, the prognosis is not very favorable. A stationary vital capacity, even at a low level, is much better than high vital capacity showing marked fluctuations, which always signifies a labile lesion. The earlier the starting point of increase in the spirometric readings is noticed and the higher the level of the vital capacity at which it starts, the better is the prognosis for a complete respiratory recuperation.

5 The difference between the preinsufflatory and the postinsufflatory vital capacity corresponds to the amount of air introduced into the pleural cavity only at the commencement of the treatment, but, as we advance in the course of the treatment and the expansibility of the uninvolved portion of the lung begins to assert itself, the difference becomes smaller and may amount to less than one fourth of the quantity of air introduced, provided the inspiratory intrapleural pressure is left negative, allowing the functioning portion to dilate to its full capacity.

6 No larger amount than one sixth of the vital capacity of the patient should be administered at the initiation of the pneumothorax. The amounts of the subsequent inflations should also commensurate in the same way with the vital capacity, but, in accordance with differences in the preinsufflatory and postinsufflatory spirometric readings, which are constantly changing throughout the course of the treatment.

7 Those who have a high vital capacity before the initiation of the treatment, as well as patients with anatomic lesions of the first or second stage, and particularly young people show a much better respiratory recuperation of the lung than other patients do, and, if properly managed and no complications set in, may attain their full physiologic vital capacity.

8 The long series of spirometric modifications as well as the complete recuperation of the respiratory capacity of the lung is observed only in cases treated with expansile pneumothorax, but in compression pneumothorax, in which the respiratory function of the treated lung is reduced to its minimum, the vital capacity diminution is very marked, and may remain permanently at its low level if fibrosis and atelectasis preponderate in the compressed lung, which is unable to reexpand. A small localized pneumothorax, because of strong pleural adhesions, has hardly any effect on the vital capacity.

9 Fluid should be aspirated as soon as possible from the pleural cavity to avoid permanent reduction of the respiratory capacity, should be partially replaced by air, and the patient made to blow the spirometer a few times daily. This effects a gradual reexpansion of the atelectatic lung and a progressively increasing vital capacity. No untoward results have been noticed in any of the patients from frequent blowing of the spirometer.

DREYER'S TUBERCLE ANTIGEN

EXPERIMENTS SHOWING THE FAILURE OF THE ANTIGEN TO PROTECT GUINEA-PIGS AGAINST EXPERIMENTAL TUBERCULOSIS [†]

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The experiments herein reported were undertaken as a preliminary investigation of the problem of the possible efficacy of Dreyer's tubercle antigen in the treatment of guinea-pigs infected with tuberculosis. It was planned to continue the experiments on a larger scale, using a much greater number of animals inoculated with material of varying grades of infectivity, and employing an adequate series of controls. The result of this preliminary study was so discouraging, however, that it was not felt that the labor and expense of an elaborate investigation would be justified. Dreyer's paper attracted considerable attention and yet there has been a great paucity of either confirmatory or contradictory reports by other workers, because of this it was felt that our negative results should be recorded.

Dreyer,¹ in 1923, asserted that, as a general rule, most of the failures in vaccine therapy have been with the acid-fast and the gram-positive organisms. Since acid-fastness and gram-positiveness have been associated with the presence in the bacterium of certain waxy, fatty or lipid substances, it would seem quite probable that the latter prevent the escape of the antigen from the organism and thus block the sole adequate stimulus for the immunity reaction. Therefore, Dreyer devised a method for removing the lipoidal substance from the tubercle bacillus and rendering the organism nonacid-fast. Tubercle bacilli grown on glycerin broth are treated repeatedly with formaldehyd and then extracted several times with acetone in a Soxhlet apparatus. The insoluble residue is dried and ground, and the organisms are tested as to their acid-fastness. If this quality persists, it is necessary to repeat the entire procedure. Often this must be done three or four times before the bacilli become nonacid-fast. This method involves rough handling of the organisms, and it might be thought that their

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1 Dreyer, G. Some New Principles in Bacterial Immunity, Their Experimental Foundation, and Their Application to the Treatment of Refractory Conditions, Brit J Exper Pathol 4 146 (June) 1923

chemical make-up is thereby seriously altered. On subjecting *Bacillus typhosus* to the same process, however, Dreyer found that this organism was still able to cause the production of immune substances. With the nonacid-fast tubercle bacilli produced by this process, termed "defatted" antigen or "diaplyte" antigen, Dreyer reported that he was enabled to produce specific precipitins, agglutinins and complement fixing bodies in normal rabbits.

With a view to study the therapeutic effects of his defatted tubercle antigen on animals suffering from well defined tuberculosis, Dreyer treated four infected guinea-pigs, three of them being the sole survivors of a group of twenty-five animals that had been inoculated with a strain of human tubercle bacilli that had caused death in the other twenty-two animals in from four to ten months. It must be borne in mind that this procedure gave Dreyer the most resistant guinea-pigs in the lot, hence, the twenty-two animals that had died cannot be considered as controls. The experiment was performed on selected animals that had already manifested a relative degree of resistance to tuberculosis, and there were no real controls.

At the time when treatment was begun, all Dreyer's animals showed well marked evidences of tuberculosis. At the date of Dreyer's report, the first guinea-pig, forty-eight weeks after infection and twenty weeks after treatment was begun, was of normal weight for its age, and the inguinal glands were smaller and harder than they had been. The second animal, whose date of infection was not recorded, appeared healthy sixteen weeks after commencement of treatment, and the inguinal glands had disappeared. The third guinea-pig, fifty-five weeks after inoculation and sixteen weeks after treatment was begun, appeared improved, but was underweight and suffered from a diarrhea. The fourth animal, fifty weeks after infection and eleven weeks after treatment was begun, gave birth to young and died a few days later. At necropsy, there was a generalized tuberculosis, but all the lesions showed abundant fibrosis, with the presence of giant cells and an absence of the lymphocytic reaction seen in active tuberculosis. Dreyer believed that the histologic picture was such as to indicate a tendency to heal. From these experiments, he concluded that treatment with diaplyte antigen brings about a definite improvement, both general and local, in animals infected with *B. tuberculosis*.

As demonstrated by Baldwin and Gardner² it is necessary to observe guinea-pigs infected with tuberculosis over long periods of time before passing judgment as to ultimate recovery. Often a very sleek, well nourished animal appears to have recovered after from six to eight months,

² Baldwin, E. R., and Gardner, L. U. Reinfection in Tuberculosis. Experimental Arrested Tuberculosis and Subsequent Infections, *Am. Rev. Tuberc.* 5:429 (Aug.) 1921.

only to emaciate gradually, lose his perineal fat, develop dyspnea from gradual consolidation of the lungs, and finally die from the disease or some complication. Some of our animals died suddenly, while fat, from hemorrhage due to erosion of a large vessel by tubercles.

Krause,³ in 1919, reported experiments with a strain of tubercle bacillus, long cultivated under artificial conditions, which was so avirulent that guinea-pigs successfully overcame infection by inhalation of it. Such an organism produced a caseous reaction, but, instead of spreading from the initial foci, the process slowly underwent retrogressive changes and, in some instances, finally completely disappeared. Dreyer's guinea-pigs were infected with an organism of slight virulence, which had not passed through an animal for fifteen years.

So far as the authors have been able to ascertain, there have thus far been reported no experiments relating to the treatment of tuberculous guinea-pigs with Dreyer's antigen, with the exception of Kettle's⁴ observations and Caulfield's⁵ recent article. Kettle, in his experiments on guinea-pigs, was unable to demonstrate any curative action of Dreyer's antigen. In fact, there was much evidence to suggest that the antigen had an adverse action on the guinea-pig treated with it, even in comparison with bacillary emulsion.

Caulfield treated six tuberculous guinea-pigs with weekly injections of 0.5 c.c. (0.1 mg. of dried bacilli) of Dreyer's antigen and gave four additional animals both prophylactic and therapeutic injections. There were four controls. All fourteen animals died of generalized tuberculosis. The average length of life in the controls was ten and a half weeks, in the treated guinea-pigs, ten weeks, in the animals receiving both prophylaxis and treatment, thirteen and seven-tenths weeks.

AUTHORS' EXPERIMENT

The antigen used by us, in this experiment, was prepared strictly in accordance with Dreyer's technique from the Board of Health Strain 305 of human tubercle bacilli, grown on glycerin broth. This was known to be an organism of medium virulence. We were able to verify the fact that the process described by Dreyer renders tubercle bacilli nonacid-fast, although it was necessary to subject the organisms four times to formaldehyde action and acetone extraction in order to bring about this result.

3 Krause, A. K. Studies on Tuberculous Infection, I, A Note on Experimental Tracheobronchial Node Tuberculosis Together with a Brief Consideration of Several Phases of Tuberculous Infection Suggested Thereby, *Am Rev Tuberc* **3** 1 (March) 1919.

4 Kettle, E. H. The Criteria of Cure in Tuberculosis of the Guinea-Pig, with Special Reference to Diaplyte Antigens, *Lancet* **1** 68 (Jan. 12) 1924.

5 Caulfield, A. H. W. A Preliminary Report on the Clinical and Experimental Use of Dreyer's Tuberculoantigen, *Canad. M. A. J.* **14** 616 (July) 1924.

Our initial dosage was only one-fourth that employed by Dreyer, that is we used 0.025 mg of dried tubercle bacilli instead of 0.1 mg. Later in the experiment, however, we ran the dosage up to the 0.2 mg employed by Dreyer. Moreover, vaccine treatment was begun much earlier in the course of the disease, usually before the appearance of glandular enlargement, in nine of the guinea-pigs, from one to three vaccine injections were made before infection. The smaller dosage was used with the idea that the antigen might prove to be toxic to the

Effect of Dreyer's Antigen on the Course of Experimental Tuberculosis in Twenty-One Guinea-Pigs, with Four Controls

	Guinea-Pig	Date of Inoculation	Date of Starting Treatment	Number of Injections	First Appearance of Glandular Enlargement (Days After Infection)	Length of Life After Infection (In Weeks)
Group 1 Eight healthy young guinea pigs (including two controls) inoculated subcutaneously in right thigh with 0.1 mg of Board of Health Strain 305 of human tubercle bacilli	7	9/14/23	10/23/23	17	12*	28
	24	9/14/23	10/23/23	17	17*	39
	26	9/14/23	10/23/23	10	17*	18
	19	9/14/23	9/23/23	16	12	21
	18	9/14/23	9/23/23	22	12	36
	40	9/14/23	9/23/23	16	12	21
	(Control) 15	9/14/23			15	17
	(Control) 49	9/14/23			15	26
Group 2 Eleven healthy young guinea-pigs (including one control) inoculated subcutaneously in right thigh with 0.01 mg of culture "Musola" (from sputum of active case of pulmonary tuberculosis) of human tubercle bacilli	31	9/29/23	9/22/23	16		17
	6	9/29/23	9/22/23	16		24
	23	9/29/23	9/22/23	20		24
	41	10/ 5/23	9/22/23	20		24
	35	10/ 5/23	9/22/23	16		16
	45	10/ 5/23	9/22/23	11		10
	158	10/ 5/23	10/23/23	12		18
	50	10/ 5/23	10/23/23	15		23
Group 3 Six healthy young guinea pigs (including one control) inoculated subcutaneously in right thigh with 0.01 mg of culture "Ravenol" of virulent bovine tubercle bacilli	388	10/ 5/23	10/23/23	15		22
	220	10/ 5/23	10/23/23	12		18
	(Control) 30	9/29/23				19
	8	10/5/23	9/22/23	10		8
	9	10/5/23	9/22/23	10		8
	47	10/5/23	9/22/23	10		8
	36	10/5/23	10/23/23	4		6
	20	10/5/23	10/23/23	4		7
	(Control) 10	10/5/23				7

All the guinea pigs, both treated and controls, died of generalized tuberculosis. The initial dosage was 0.025 mg, the maximum dosage, 0.2 mg (in terms of mass of dried tubercle bacilli).

* Glandular enlargement appeared before antigen treatment was started. In the three treated animals, adenopathy appeared earlier than in the five controls.

animals, and that it would be to their advantage to start early with small amounts, which would be gradually increased. This procedure seemed justified by subsequent developments, in that there was earlier adenopathy in the treated animals and a more rapid loss of weight in the guinea-pigs receiving full doses.

The vaccine was injected into the subcutaneous tissue of the abdomen. Injections were made weekly, on several occasions, two weeks were allowed to elapse. Two doses each of 0.025 mg, then five doses of 0.05 mg, then six doses of 0.1 mg, then three doses of 0.2 mg were given. The remaining doses were of 0.1 mg.

The salient features of the experiment are indicated in the accompanying table. Twenty-five guinea-pigs, weighing between 280 and 400 gm., were inoculated with living tubercle bacilli, of which group twenty-one were treated with the antigen, four serving as controls. In nine instances, vaccination was begun before the animals were inoculated. The animals of Group 1, comprising six treated guinea-pigs and two controls, were inoculated subcutaneously in the right thigh with 0.1 mg. of Board of Health Strain 305 of human tubercle bacilli. Those of Group 2, comprising ten treated animals and one control, were infected with 0.01 mg. of a strain of human tubercle bacilli cultured by us from a patient with active pulmonary tuberculosis. The animals of Group 3, comprising five treated guinea-pigs and one control, were inoculated with 0.01 mg. of a culture of virulent bovine tubercle bacilli. The animals were weighed at frequent intervals, but as their weight curves do not add much of interest, we have dropped such data from the tabulation. We found, as did Dreyer, that the larger doses of vaccine (0.2 mg.) often caused rapid loss of weight from which the animal would recover if treatment was suspended for a couple of weeks.

All the twenty-five animals employed in this experiment died with postmortem evidences of generalized tuberculosis, and there was no apparent difference in the course of the disease between the treated animals and the controls. As a matter of fact, two conditions observed would indicate that the antigen is in itself either toxic or sensitizing: (1) There was a tendency for earlier glandular enlargement in the treated guinea-pigs, and (2) cessation of vaccine treatment in emaciated animals would often be followed by considerable gains in weight. By referring to Group 1 of the table, it will be noted that in the three starred animals glandular enlargement appeared before vaccine treatment was begun. If we take these three animals, together with the two which at no time received treatment, as controls, it will be observed that adenopathy appeared earlier in the vaccinated than in the untreated animals.

CONCLUSION

A study of the course of the disease in a group of twenty-five guinea-pigs experimentally infected with tuberculosis, of which group twelve received therapeutic vaccination with Dreyer's tubercle antigen and nine both prophylactic and therapeutic vaccination, four animals serving as controls, failed to adduce any evidence that this antigen protects guinea-pigs against tuberculosis, prolongs life, or in any way favorably influences the course of the disease.

INULIN AND ARTICHOKES IN THE TREATMENT OF DIABETES^{*}

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INTRODUCTION

The use of Jerusalem artichokes, the edible vegetable in which inulin is most commonly found, in the diabetic diet has interested students of the disease ever since Kulz¹ and Bouchardat² recommended it. Kulz and Bouchardat believed that inulin was utilized better than starch, but von Noorden³ stated that this would not hold true with the severe cases in the same degree as with the mild ones. Sandmeyer,⁴ Persia⁵ Lewis⁶ and Goudberg⁷ noted that inulin, when fed by mouth, appeared either not at all or in small amounts in the stools in the normal man. Sandmeyer, however, recovered in the stools more than half the inulin given to a dog, whose pancreas had been removed. Goudberg, who reviewed the subject exhaustively, gave from 100 to 200 gm of inulin to diabetic subjects, and observed a pronounced rise in the respiratory quotient and in the metabolism, persisting for from six to ten hours afterward in some cases. He therefore concluded that it was well utilized. Offenbacher and Elhassow⁸ and Dumont⁹ also found inulin in the form of artichokes useful in the diabetic diet. Mendel,¹⁰ however, was unable to find any ferment or enzymes in the body which would hydrolyze the inulin, and therefore held that the good results of inulin feeding were

^{*} From the Diabetic Clinic of the New England Deaconess Hospital.

[†] This paper is No. 46 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed in part by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases.

1 Kulz. Beitr. z. Path. u. Therap. der Diabetes, *Jahrb. Tierchem.* **4**, 448, 1874.

2 Bouchardat, quoted by von Noorden, in *Von Leyden's Handbuch des Ernährungs Therapie* **2**, 227, 1904.

3 Von Noorden (Footnote 2).

4 Sandmeyer, W. *Ztschr. f. Biol.* **31**, 32, 1895.

5 Persia. *Nuova Rivista Clin. Therapeut.* **8**, 1905, *Jahrb. Tierchem.* **25**, 822, 1905.

6 Lewis, H. B. The Value of Inulin as a Foodstuff, *J. A. M. A.* **58**, 1176 (April 20), 1912.

7 Goudberg. *Ztschr. f. exper. Path. u. Therap.* **13**, 310, 1913.

8 Offenbacher, R., and Elhassow, W. *München med. Wchnschr.* **69**, 1508 (Oct. 27), 1922.

9 Dumont. *Bull. Acad. de med.* **87**, 721 (June 27), 1922.

10 Mendel, L. B. *Zentralbl. f. d. ges. Physiol. u. Path. d. Stoffwechs.* **3**, 641, 1908.

due to its nonabsorption Lewis,⁶ Chittenden,¹¹ Bierly¹² and others recognized that hydrolysis of inulin by the hydrochloric acid of the stomach would liberate some levulose Neubauer¹³ found no increase in the excretion of levulose in a case of levulosuria after the giving of inulin, and Lewis and Frankel¹⁴ obtained no increase in glycosuria in phloridzinized animals after inulin feeding The recent studies of Okey¹⁵ have thrown new light on the behavior of inulin, as she has shown that not only is inulin hydrolyzed by the hydrochloric acid of the stomach but also that an inulase may be isolated from the feces of normal subjects The nature and source of this inulase is not explained It may be of bacterial origin, such as occurs in *Penicillium glaucum* and *Aspergillus niger* The latter has been studied by Grafe and Vouk¹⁶ Green,¹⁷ Bourquelot¹⁸ and Dean¹⁹ have found inulase in various plants Green states that the enzyme inulase occurs only in the germinating tuber In the resting tuber, the enzyme occurs as a zymogen from which it may be released by the action of "warmth" or under conditions of mild acidity The enzyme hydrolyzes inulin very slowly, some of his experiments extending over a period of six days It is completely destroyed by boiling and by the action of 0.21 per cent hydrochloric acid at 40 C

The chemical nature of inulin has been the subject of many investigations Dean²⁰ and Willaman²¹ regard inulin as a mixture of anhydrides of levulose of varying sized molecules In artichoke tubers, a series of inulin-like substances (such as inuloids and inulids), different in solubilities but all anhydrides of levulose, is found, some of which may be more easily hydrolyzed than others True inulin is defined by Dean as the carbohydrate mixture precipitated in cold alcohol of 60 per cent and with a specific rotation of $[\alpha]_D = -30^\circ$ to -40° Colin²² has studied the occurrence of inulin in the tuber of the Jerusalem artichoke, and finds that the leaves elaborate reducing sugars which are condensed in the stem to inulin and stored in the tubers During the winter months, inulin is converted back into reducing sugars and intermediate products are formed which are more and more readily hydrolyzed as this transformation proceeds Thus, in the Jerusalem arti-

11 Chittenden, R. H. Am J Physiol **2** 17, 1898

12 Bierly, H. Compt rend Acad d sc **150** 116, 1910, Compt rend Soc de biol **59** 256, 1905, Biochem Ztschr **44** 402, 1912

13 Neubauer. Munchen med Wchnschr., 1905, p. 1525

14 Lewis and Frankel. J Biol Chem **17** 365, 1914

15 Okey, R. J Biol Chem **38** 33 (May) 1919, *ibid* **39** 149, 1919

16 Grafe and Vouk, V. Ztschr. Garungsphysiol **3** 327, 1913

17 Green. Ann Botany **1** 223, 1888

18 Bourquelot. Compt rend Soc de biol **116** 1143, 1893

19 Dean. Botany Gaz **25** 69, 1903

20 Dean, A. L. Am Chem J **32** 69, 1904

21 Willaman, J. J. J Biol Chem **51** 275 (March) 1922

22 Colin, H. Bull. Ass. Chim. Sucr. Dis **37** 121, 1919

chokes, one may expect that a slight decrease in the amount of levulose derived from inulin would occur during storage in the winter Dr J S Caldwell has suggested that these changes may not occur so readily in artichokes stored under conditions such as to prevent freezing as when they are stored underground Table 1 gives analyses of Jerusalem artichokes sampled at intervals for nearly three months The change in sugars other than levulose was, however, very slight, as the table shows The figures for sugars are those found after hydrolysis of the inulin

Shohl²³ also has recently analyzed Jerusalem artichokes finding 15 per cent carbohydrate content He found the vitamin water-soluble B present in scant amounts Desgrez, Bierry and Rathery²⁴ have recommended the administration of this vitamin with levulose

TABLE 1—*Summary of Analyses of Artichokes from River Ridge Farm, Franklin, Pa*

Date Received	Levulose	Total Solids in Juice	Total Reducing Sugars	Glucose by Iodin	Ratio of Levulose to Total Sugar
Nov 9, 1923	12.4		17.2	4.5	0.778
Nov 17, 1923	11.7		14 "	3.6	0.796
Nov 27, 1923	11.8	18.6			
Dec 3, 1923	12.6	18.6	15.4	4.5	0.818
Dec 29 1923	12.5	20.0	15.7	4.0	0.794
Jan 7, 1924	11.2	17.6	13.3	3.6	0.844
Jan 17 1924	10.6	18.4	14.4		0.732
Jan 28 1924	11.5	18.9	14.7		0.782
Jan 31, 1924	10.4	18.6			

* The analyses were made at the Bureau of Standards, Department of Commerce, Washington, D. C., and were furnished us by Joseph C Sibley with the permission of F. A. Bran, acting director, and George K. Burgess, director

OBJECTS AND METHODS OF THE INVESTIGATION

Results of previous studies have not agreed as to the food value of inulin Failure to recover inulin in the stools, the well known hydrolysis of inulin by dilute hydrochloric acid and the increase in the respiratory quotient after inulin feeding reported by one observer have been offset by the absence of a demonstrable inulase in the digestive juices The question of the usefulness of artichokes in the diabetic diet is a subject requiring study from many angles and is not to be answered by experiments with a single type of inulin extracted from plants, although such experiments may be expected to give helpful information One must bear in mind (1) the possible hydrolysis of inulin in the stomach and in the intestine, (2) the possible occurrence in the artichoke of anhydrides of levulose intermediate between "inulin" and levulose, and of levulose itself, (3) the existence of an inulase in

23 Shohl, A. T. J. Am. Chem. Soc. 45:2754, 1923

24 Desgrez, Bierry and Rathery. Bull. Acad. de med. 88:167 (Oct. 31) 1922

artichokes, (4) the occurrence of small amounts of glucose and sucrose, and (5) the various effects produced by different types of cooking

Our studies have been both clinical and experimental. They were inspired by the work described above, the investigations on levulose at the Nutrition Laboratory of the Carnegie Institution and in this clinic,²⁵ and by the beneficial effects observed with artichokes in our own patients. Clinically, we have used Jerusalem artichokes, inulin and levulose, by adding these foods to the diet, by substituting them for other carbohydrate foods in the diet and by replacing inulin with artichokes. We have relied on changes in the respiratory quotient and heat production as the chief evidence of absorption and utilization of carbohydrate ingested. Metabolism experiments have been carried on by giving the patients in the fasting state varying amounts of artichokes, inulin or levulose and observing changes in the respiratory metabolism, blood and urine during the following six hours. The Tissot apparatus with modified Haldane gas analysis apparatus was used. The figures recorded for the respiratory quotient and the heat production represent averages for three consecutive periods of ten minutes. The inulin employed was prepared from dahlia tubers and provided through the courtesy of Dr J. S. Caldwell of the United States Department of Agriculture, Washington, D. C. It contained 2.5 per cent reducing sugar, which was almost entirely levulose. The artichokes, *helianthus tuberosus albus*, were obtained through the courtesy of Joseph C. Sibley of River Ridge Farm, Franklin, Pa.

EFFECT OF A SINGLE PORTION OF ARTICHOKE ON SIX DIABETIC PATIENTS

The changes in metabolism in six diabetic patients who received baked Jerusalem artichokes in the postabsorptive state are shown in Table 2. All were moderately severe cases under treatment with insulin. This was omitted sixteen hours before and during the test. On the day of the experiment, a preliminary basal metabolism of three ten minute periods was used. The artichokes were freshly baked in their skins and served warm with a little salt and 200 c. c. of Kaffee Hag flavored with saccharin. Benedict and Benedict²⁶ showed that caffeine-free coffee given in this manner did not affect the metabolism. It was intended to give 1.5 gm. of carbohydrate per kilogram of body weight, but variations in the patients' appetites made it possible to try doses of varying amounts.

²⁵ Joslin, E. P. *Diabetic Metabolism with High and Low Diets*, Carnegie Inst., Washington, Pub. 323, 1923.

²⁶ Benedict, C. G., and Benedict, F. G. *Boston M. & S. J.* **188**: 849 (May 31) 1923.

TABLE 2—*Effect of Baked Antichokes on the Metabolism of Six Diabetic Patients**

Case	Carbohydrate Given		Weight of Patient Above or Below Standard, per Cent	Variation of Basal Metabolism from Standard (H & B), per Cent		Half Hours After Ingestion												
	Gm	Grams per Kg				Basal	1	2	3	4	5	6	7	8	9	10	11	12
1	77	2.0	-39	-3	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.76 1,081	0.84† 1,222 +13		0.81 1,187 +10				0.88 1,185 +10			0.87 1,210 +10		
2	79	1.1	-7	-9	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.77 1,420	0.74 1,586 +12	0.83 1,540 +9				0.83 1,484 +5				0.82 1,466 +3		
3	37	0.7	-6	+3	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.71 1,409	0.71 1,409 ±0	0.71 1,368 -4		0.81 1,135 +2						0.80 1,390 -1		
4	51	10.9	-7	+1	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.76 1,321	0.75 1,385 +5	0.77 1,434 +9		0.79 1,393 +5						0.81 1,365 +3		
5	81	1.5	-10	+10	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.73 1,173	0.69 1,592 +8	0.78 1,637 +11				0.77 1,523 +3				0.76 1,505 +2		
6	53	1.2	-26	+1	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.82† 1,701	0.77 1,324 +1	0.82 1,334 +3				0.83 1,352 +4				0.94 1,303 ±0		

* Since some of these cases will be mentioned in other papers, for purposes of identification their personal case numbers are given. Case 1, Joslin 3692; 2, Joslin 2820; 3, Joslin 3666; 4, Joslin 3728; 5, Joslin 3687; 6, Joslin 1776; 7, Joslin 3565; 8, Joslin 2917; 9, Joslin 2977; 10, normal; 11, Joslin 3692; 12, normal; 13, Joslin 3129; 14, Joslin 3640; 15, normal; 16, Joslin 3191.

† Respiratory quotient 0.82 two days before

‡ Maximum, 0.88

The most striking feature observed was a pronounced rise in the respiratory quotient which occurred uniformly and tended to reach its maximum in from two to six hours. Of these patients, in two instances the maximum respiratory quotients were obtained during the sixth hour, and in three cases quotients within 0.01 of the maximum quotient were obtained in the sixth hour. In two cases, the maximum quotients were obtained in the third and fourth hours. The patients whose weight was most below standard exhibited the greatest increases in respiratory quotient. The amount of carbohydrate taken per kilogram of body weight also influenced the rise in respiratory quotient. The average maximal increase in respiratory quotient was 0.076.

The average maximal increase in heat production was 8.5 per cent. This may be compared with an average maximal increase of 17 per cent after levulose recorded by Joslin²⁷ in the Carnegie series, and of 10 per cent in three mild cases shown in Table 201.²⁸ The increase in heat production was clearly related to the amount of artichokes taken. Thus Cases 1, 2 and 5 (Table 2) received an average of 83.6 gm. of carbohydrate, with a resultant average increase in metabolism of 12 per cent. Cases 3, 4 and 6 received an average of 47 gm. of carbohydrate, with a resultant average maximal increase in metabolism of 5 per cent.

If we compare these results with the effect of levulose alone on the metabolism, it is seen, in Table 3, that the highest quotients after levulose are obtained within two hours after its ingestion. This is even more apparent in Table 201 of Joslin's book,²⁸ in which, with one exception, all maximal quotients were obtained within the first two hours after the taking of levulose. The slower and more prolonged effect on the respiratory quotient after the artichokes suggests slower liberation of carbohydrates and slower absorption. In one case, Case 1, a comparison is afforded between the results after artichokes and after levulose. Here, in both instances, the rise in quotients persisted throughout the six hours. The average increase in respiratory quotient was 0.098 with artichokes as against 0.025 with levulose. Similarly, the average increase in heat production was 11 per cent with artichokes and 11 per cent with levulose. The maximal increase in respiratory quotient was obtained during the second hour with levulose and during the fifth hour with artichokes.

Diabetic subjects whose metabolism is below standard exhibit a greater increase in respiratory quotient after taking levulose than do those with higher basal metabolism. One may say that a diabetic patient

27 Joslin, E. P. *The Treatment of Diabetes Mellitus*, Philadelphia, Lea and Febiger, 1923, p. 543.

28 Joslin (Footnote 27, p. 292).

TABLE 3—*Effect of Levulose on the Metabolism of Three Diabetic Patients*

Case	Amount Given		Weight of Patient Above or Below Standard, per Cent	Variation of Basal Metabolism from Standard (H & B), per Cent		Half Hours After Ingestion											
	Total, Gm	Grams per Kg				Basal	1	2	3	4	5	6	7	8	9	10	11
7	50.6	1.6	-40	-22	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.81 804	0.82 825 +3	0.95 933 +16	0.86 906 +13			0.80 899 +12			0.84 873 +9		
8	87	1.6	-24	-10	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.76 1,670	0.80* 1,977 +18		0.74 1,729 +4			0.79 1,490 -11			0.78 1,554 -7		
1	48	1.2	-39	-3	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.77 1,070	0.79 1,172 +10	0.80 1,237 +16				0.79 1,172 +10			0.80 1,141 +7		

* Maximum for 1 single period, 0.83

TABLE 4—*Effect of Levulose in Repeated Small Doses*

Case	Amount per Hour, Gm	Hours	Weight of Patient Above or Below Standard, per Cent	Variation of Basal Metabolism from Standard (H & B), per Cent		Half Hours After Ingestion											
						Basal	1	2	3	4	5	6	7	8	9	10	11
9	12	6	-19	-1	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.79 1,641	0.78 1,664 +1	0.76 1,663 +1	0.76 1,745 +6	0.76 1,745 +6	0.77 1,749 +7	0.77 1,749 +7	0.75 1,795 +9	0.80 1,715 +5			
6	10	5	-26	+0.4	Respiratory quotient Calories per 24 hours Percentage deviation from basal	0.82 1,327	0.79 1,296 -2	0.87 1,337 +1	0.87 1,337 +1	0.82 1,397 -5	0.82 1,397 -5	0.83 1,247 -6	0.97 1,364 +3				

must have a subnormal metabolism in order to react to levulose like a normal subject

Another comparison between levulose and artichokes is presented in Table 4, which gives the effect on the metabolism of levulose in repeated small doses. These two subjects received levulose, in one instance at the rate of 10 gm an hour, and in the other instance, 12 gm an hour. Case 9 was a young man, aged 19, with fairly severe diabetes, who did not utilize levulose well. Case 6 is of special interest because results are at hand both after a single administration of artichokes and after levulose given in repeated doses. He had a mild case and utilized both the levulose and artichokes very well. In each experiment, the maximal respiratory quotients were obtained at the end of the six hours. The increases in heat production were greater in Case 9, although in this case there was less increase in respiratory quotient. The curves in blood sugars differed. In Table 6, it is seen that the blood sugar percentages rose steadily during the six hours during which levulose was given, whereas, after the single dose of artichokes, the blood sugars rose to a maximum at the end of two hours and decreased at the end of six hours. In general, the similarity of results obtained after levulose and after artichokes is striking.

It must be remembered that one-fifth the available carbohydrate in these artichokes was dextrose. The amounts of dextrose given varied between 13 and 20 gm in the different experiments. Possibly this was better utilized by reason of slower absorption.

EFFECT OF INULIN ON THE METABOLISM OF DIABETIC PATIENTS

The effect of inulin on the metabolism of three normal subjects and two diabetic patients is shown in Table 5. In the instance of the three normal subjects, two females and one male, a difference in the results appears. The first two patients, Cases 10 and 11, showed very slight increases in respiratory quotients but fairly definite increases in heat production through the six hours. In Case 10, intestinal fermentation was noticed about three hours after the inulin was taken, and inulin was recovered in the stools eight hours later. In Case 12, a marked rise in respiratory quotient occurred during the second half-hour which also was found persisting during the fifth half-hour. It is true that she seemed a little nervous, but she had practiced wearing the mask for the metabolism determination the day before and was a very intelligent and cooperative dietitian. The results represent averages of six periods in all of which the quotients were between 0.90 and 0.93. It is very difficult to justify the exclusion of these results. That such variations in pure inulin, due to varying degrees of hydrolysis in the stomach, are to be expected, was the opinion of Lewis. Furthermore, other factors, such as the character of the bacterial flora, might be expected to affect

TABLE 5—Effect of Inulin 15 Gm per Kilogram, on Metabolism of Three Normal and Two Diabetic Subjects

Case	Amount Gm.	Weight of Patient Above or Below Standard, per Cent.	Variation of B is il Metabolism from Standard (H. & B.), per Cent.		B is il	Half Hours After Ingestion											
						1	2	3	4	5	6	7	8	9	10	11	12
10 (norm il)	105	—1	—1	Respiratory quotient Calories per 24 hours Percentage deviation from b is il	0.80 1.708		0.80 1.777 +4		0.81 1.781 +1				0.80 1.797 +5				0.79 1.786 +5
11 (norm il)	92	±0	—12	Respiratory quotient Calories per 24 hours Percentage deviation from b is il	0.80 1.258		0.79 1.310 +4			0.81 1.282 +2				0.83 1.331 +6			0.79 1.345 +7
12 (norm il)	60	—26	+12	Respiratory quotient Calories per 24 hours Percentage deviation from b is il	0.83 1.379		0.91 1.294 —5			0.91 1.265 —7				0.82 1.295 —7			0.83 1.282 —6
13 (diabetic)	90	—5	—1	Respiratory quotient Calories per 24 hours Percentage deviation from b is il	0.77 1.550		0.77 1.605 +6		0.76 1.547 ±0		0.71 1.464 —6				0.77 1.544 —0.4		0.75 1.685* +9
14 (diabetic)	90	—13	—8	Respiratory quotient Calories per 24 hours Percentage deviation from b is il	0.77 1.379		0.77† 1.430 +3		0.48 1.403 +2		0.77 1.475 +7				0.76 1.321 —4		

* Restless

† Maximum, 82

the absorption of split products of inulin. The three normal subjects exhibited slight increases in respiratory quotient and, in two of the cases, increases in heat production occurred. The fact that the respiratory quotients and metabolism were not below the initial levels at the end of the six hours may be taken as evidence that absorption of some food had occurred. With the two diabetic patients, the case is somewhat different. Both cases were severe. Patient 13 had just recovered from a period of severe acidosis, and was restless during the sixth hour. During the third hour, drowsiness may account for the fall in respiratory quotients. Patient 14 had a single quotient of 0.82 during the second half-hour, but the average quotients during this half-hour did not exceed the basal quotient. In both patients, a slight increase in heat production occurred, but the experiments do not otherwise give evidences of absorption of inulin.

TABLE 6—*Effect of Inulin, Levulose and Artichokes on the Blood Sugar*

Case	Substance Given	Amount Carbohydrate, Gm	Fasting	Hours After Ingestion					
				1	2	3	4	5	6
12 (normal)	Inulin	60	0.09		0.08				0.08
11 (normal)	Inulin	92	0.08		0.08				0.05
10 (normal)	Inulin	105	0.10	0.10		0.09			0.10
14 Diabetic	Inulin	90	0.20	0.21		0.22		0.17	
13 Diabetic	Inulin	90	0.30		0.31	0.28			0.23
7 Diabetic	Levulose	50.6	0.07		0.08		0.09		0.09
8 Diabetic	Levulose	87.0	0.33		0.44				0.30
1 Diabetic	Levulose	48.0	0.24	0.31		0.33			0.24
9 Diabetic	Levulose	72.0*	0.16	0.17		0.27		0.28	0.24
6 Diabetic	Levulose	50.0†	0.17	0.16			0.22		0.25
1 Diabetic	Artichokes	71.0	0.25		0.30				0.21
2 Diabetic	Artichokes	79.0	0.19	0.27		0.24			0.17
3 Diabetic	Artichokes	37.0	0.26			0.29			0.23
4 Diabetic	Artichokes	51.0	0.17		0.19	0.17			0.13
5 Diabetic	Artichokes	81.0	0.31	0.33		0.29			0.20
6 Diabetic	Artichokes	53.0	0.19	0.22		0.26			0.24

* Levulose given, 12 gm. an hour

† Levulose given, 10 gm. an hour

BLOOD SUGAR, AS AFFECTED BY ARTICHOKES, INULIN AND LEVULOSE

The changes in blood sugar percentages after the administration of inulin, levulose and artichokes are shown in Table 6. Inulin produced no significant increase in either the normal or the diabetic subjects. With levulose the facts are quite different. With the exception of the remarkable Case 7, in which the patient showed almost no change in the blood sugar level after taking 1.5 gm. of levulose per kilogram of body weight, levulose produced a distinct rise in the percentage of blood sugar of diabetic patients, whether taken in a single large dose or as small amounts at hourly intervals. In the latter instance, the increase in the blood sugar tended to be progressive so long as levulose was taken. When baked artichokes were given to diabetic patients, a slight rise in blood sugar was observed in every instance, with a fall at the end of the six

hours to values slightly lower than the initial value. In two cases, we are able to compare in the same subject the results with levulose and with artichokes. With Case 1, the increase in blood sugar was 0.05 when 71 gm of carbohydrate in the form of baked artichokes was given, whereas, when 48 gm of pure levulose was taken, the increase during the first hour was 0.07. At the end of six hours after artichokes, the blood sugar was below the initial level, whereas it was the same as the initial level in the levulose test. In Case 1, the increase in respiratory quotient was greater with artichokes and more prolonged than with the levulose. With Case 6, a comparison is possible between the effect on the blood sugar of levulose taken in hourly doses and artichokes. The greater increase in the blood sugars occurred after the levulose. In

TABLE 7—*Effect of Inulin Obtained from Dahlia Bulbs on the Sugar of the Blood and Urine Before and After Hydrolysis*

Subject	Time	Blood Sugar per 100 C c			Corpuscles, per Cent by Volume	Urine		Remarks
		Whole Blood, Mg	Plasma, Mg	Cor- puscles, Mg		Volume per Hour, C c	Sugar per Hour, Mg	
15 (Normal) Weight, 57 kg Age, 26	8 06	109+6	117+1					
	8 27					38		Fasting
	8 35	Inulin 95 gm in 500 c c of water						
	9 35	109+6	107+4			53		
	10 43	109+0	105+7			131		
	2 35	101+6	98+3			9		
16 (Diabetic)	8 30					27	121+1	Fasting
	8 55	263+7	270+0	234+20	35			
	9 15	Inulin, 70 gm in 600 c c of water						
	9 50					42	252+4	
	10 32	263+13	267+0	255+22	38			
	10 47					142	415+0	
	11 18	282+4	280+2	286+8	36			
	11 56					200	513+0	
	3 34	231+7	238+7	218+7	34			
	3 42					75	162+3	

both cases, high quotients were obtained at the end of the period. These results with the respiratory quotient showed that (1) the inulin used was not absorbed, or was absorbed unchanged, or was absorbed too slowly to affect the blood sugar, (2) baked artichokes did not increase the blood sugar as much as pure levulose in equivalent amounts, (3) when levulose was given in small divided doses, the effect on the blood sugar was similar to that in the experiments in which artichokes were taken, but a greater increase was found, and (4) the absorption of some reducing sugar, after the taking of artichokes was proved.

Inulin made no change in the urinary sugar excretion, with the possible exception of in Case 13, a case of severe diabetes in which sugar excretion may have increased for another reason, namely, the omission of insulin on that day. One diabetic patient excreted no sugar after either levulose or artichokes. One patient excreted no sugar after levulose, and another was sugar-free during the six hours after taking

artichokes The others showed definite increases in glycosuria after both levulose and artichokes Levulose was not found in the urine by the Seliwanoff test Patient 1 excreted 8.5 gm of sugar in six hours after 48 gm of levulose, and only 9.2 gm of sugar after 71 gm of carbohydrate in the form of baked artichokes

ABSORPTION OF INULIN

Two experiments were carried on to determine whether there was any evidence of the absorption of inulin as such or of some polymer of levulose The method employed was to give inulin by mouth and at intervals to determine the venous blood sugar before and after hydrolysis of the Folin filtrate For this purpose it was found that two drops of the 1 per cent hydrochloric acid, when added to 2 c c of the Folin filtrate and set in a boiling water bath for two hours, would hydrolyze inulin without causing destruction of the glucose normally present Stronger solutions of hydrochloric acid, such as 10 per cent, could not be used since they caused destruction of the glucose as well as hydrolysis of inulin The added acid was then neutralized with 2 drops of a sodium hydroxide solution, which was equivalent to the hydrochloric acid Similarly, the sugar in the urine was determined before and after hydrolysis by the method of Folin In Table 7 are shown the results It is seen that, with the normal subject, the difference value, i. e., the increase in reducing power of the Folin filtrate as hydrolyzed with hydrochloric acid, increased somewhat after the taking of inulin However, we were unable to get a Seliwanoff test In the case of the diabetic subject, there was a considerable increase in the difference value in the urinary excretion found, but in neither case could a Seliwanoff reaction be obtained These results are suggestive but not conclusive It is to be remembered that the blood specimens were venous blood, and hence it is possible that absorbed inulin was held in the liver and merely displaced dextrin-like substances Another possibility is that it was taken up by the tissues In either case, it would not be demonstrable in the venous blood

INULIN IN THE PREVENTION OF ACIDOSIS

The tendency toward the development of acidosis, which might have been produced by the withdrawal of insulin on the experiment days, was apparently counteracted by all three substances The plasma carbon dioxide combining power remained practically constant during the six hours of the inulin tests, increased by 1.9 per cent by volume during the levulose experiments, and, in the experiments with artichokes increased from an average of 56.8 per cent by volume fasting to 57.7 per cent by volume at the end of six hours

In two instances, acidosis was present when the artichokes were taken. The fasting urine specimen of Case 5 gave a +++ ferric chlorid test and the plasma carbon dioxid combining power was only 44.3 per cent by volume. The fasting urine of Case 3 gave a ++ ferric chlorid test. In the first instance, the respiratory quotient rose from 0.73 to 0.78, the heat production increased by 11 per cent, and the plasma bicarbonate increased by 1 per cent by volume during the two hours following ingestion of artichokes. In the second case, a rise of 0.07 in the respiratory quotient and an increase of 2 per cent by volume in the plasma bicarbonate occurred during the third hour. Thus, the beneficial effects of artichokes on diabetic acidosis were clearly suggested.

TABLE 8—*Alterations in Plasma Cholesterol*

Carbohydrate	Case	Grams per Kg	Milligrams per 100 C c			
			Fasting	Hours After Ingestion		
				1	2	6
Inulin	Normal 10	1.3	96.3	97.7	124	159
	Normal 11	1.5	114		151	155
	Normal 12	1.3	116	112		106
Inulin	13	1.5	112	161	188	117
	14	1.5	426	452	452	469
Levulose	7	1.6	114	106	95.7	102
	8	1.6	122		110	116
	1	1.2	152	126	120	192
	9	1.3	96.3	106	116	161
	6	1.1	217	227	231	189
Artichokes	1	1.8	135	188		210
	2	1.4	120	145	140	114
	3	0.7	109		115	134
	4	0.9	63.4	65.2	81.6	
	5	1.5	102	104	141	169
	6	1.2	215	147	169	194

BLOOD LIPOIDS, AS AFFECTED BY ARTICHOKEs, INULIN AND LEVULOSE

In Tables 8 to 11 are given analyses of the plasma lipoids, for which we are indebted to Prof. W. R. Bloor of the University of Rochester Medical School and the Robinson Foundation.

Temporary increases in the blood lipoids are observed after fat feeding, starvation, narcosis and hemorrhage. In any lipemia, increases occur in the fatty acid, lecithin and cholesterol although the latter is inconstant in alimentary lipemia. The rate of fat absorption and the rate of disappearance from the blood control alimentary lipemia. Bloor²⁹ considers that diabetic lipemia is due to the lack of hormone aiding in the removal of fat, an hypothesis apparently confirmed by the discovery of insulin. We have observed that insulin prevents the increase both in the blood sugar and the blood lipoids which follows the taking of levulose by a diabetic patient.

²⁹ Bloor, W. R. J. Biol. Chem. **49** 20, 1921

In seeking to explain the changes in plasma lipoids in these experiments, three possible sources must be considered (1) the food, (2) the fat depots of the body, and (3) the synthesis from other constituents of the body. The mechanism of synthesis of blood lipoids from other constituents of the body or diet is entirely unknown, according to Bloor. The influence of fasting and the consequent drain of fat stores on the plasma lipoids is difficult to evaluate in these experiments. In fasting, the blood sugar falls and the blood fat increases. In the experiments with levulose and artichokes, though not with inulin, however, the blood sugar of diabetic patients rose either during the first two hours or throughout the six hours. This fact shows clearly that the

TABLE 9—*Alterations in Plasma Fatty Acid*

Carbo- hydrate	Case	Grams per kg	Milligrams per 100 C c			
			Fasting	Hours After Ingestion		
				1	2	6
Inulin	Normal 10	1.3	356	406	356	346
	Normal 11	1.5	332		314	328
	Normal 12	1.3	246	252		356
Inulin	13	1.5	446	507	570	453
	14	1.5	482	540	622	697
Levulose	7	1.6	332	324	338	320
	8	1.6	645		657	606
	1	1.2	393	411	428	442
	9	1.3	400	388	402	324
	6	1.1	348	623	673	643
Artichokes	1	1.8	402	435		522
	2	1.4	392	417	374	422
	3	0.7	398		448	418
	4	0.9	444	358	456	
	5	1.5	422	440	480	536
	6	1.2	667	623	456	450

results are not to be regarded as entirely due to fasting and nonabsorption of the substances fed. Furthermore, the subjects of levulose and artichoke experiments received amounts sufficient on the average for their caloric needs, during the period of the test. Another factor that may have influenced the results was the withdrawal of insulin. All but one of the diabetic subjects had been receiving insulin. None had been given for at least sixteen hours prior to the experiments. Only one patient, Case 5, had received as much as 30 units a day. It is unlikely that its withdrawal could have greatly affected the experimental results. We feel that the substances fed must be regarded as of chief importance in producing the observed changes.

In discussing the alterations in plasma lipoids, we shall consider each separately. Significant increases in the cholesterol of the plasma occurred in twelve of sixteen experiments. In eight instances, the maximum was reached at six hours and in the other four cases during the first two hours. The increases occurred in both normal

and diabetic subjects and in all four types of experiment. The four subjects, one normal, Case 11, and three diabetic, who did not exhibit an increase in plasma cholesterol, were distinguished from the ten who did, first, by their weight and, second, by the behavior of their respiratory quotients. Their average weight was 29 per cent below standard, and their average maximal increase in respiratory quotient during the experiments was 0.095. The average weight of the ten subjects was 11 per cent below standard, and the average maximal increase in respiratory quotient during the ten experiments was 0.04. In Cases 1 and 6, two experiments each were done. Patient 6 exhibited an increase in cholesterol when levulose was given in five hourly doses of 10 gm, but not when artichokes equivalent to 53 gm of carbohydrate

TABLE 10—*Alterations in Plasma Lecithin*

Carbo- hydrate	Case	Grams per Kg	Milligrams per 100 C c			
			Fasting	Hours After Ingestion		
				1	2	6
Inulin	Normal 10	1.3	132	157	182	186
	Normal 11	1.5	270		274	282
	Normal 12	1.3	158	198		199
Inulin	13	1.5	205	278	262	208
	14	1.5	557	541	570	515
Levulose	7	1.6	194	182	258	229
	8	1.6	262		271	286
	1	1.2	516	502	449	326
	9	1.3	230	227	381	379
	6	1.1	269	194	334	389
Artichokes	1	1.8	331	188		290
	2	1.4	438	309	336	240
	3	0.7	274		294	290
	4	0.9	232	243	243	
	5	1.5	350	285	302	272
	6	1.2	370	252	234	234

were taken. The more rapid absorption of pure levulose, as compared with artichokes, may have brought about this result. This suggests that the tendency for an increase in the plasma cholesterol to occur after the ingestion of carbohydrates is dependent on the character of the metabolism thereby induced and on the preceding nutritional state. The lower the weight and the greater the increase in respiratory quotient, the less will be the increase in plasma cholesterol, or the better the utilization of carbohydrate, the less will be the tendency for an increase in cholesterol.

The initial level of the blood sugar and of the cholesterol seemed not to affect the increase. The severity of the diabetes seemed to be a factor. Thus Cases 7, 8 and 6, who had little or no increase in plasma cholesterol, were milder than the other diabetic cases.

The alterations in the fatty acids of the plasma paralleled fairly closely those of the cholesterol. Increases occurred in thirteen experiments, the

highest values being observed in the sixth hour in seven cases, and in the second hour in five cases. The parallelism was not exact, however, for, in five instances, an increase in one lipid was not accompanied by an increase in the other. Furthermore, in only six cases, the maximal value for cholesterol occurred at the same time as that for fatty acid. Nevertheless, the outstanding fact is that, in thirteen experiments, an average maximal increase of 24.6 per cent occurred during the six hours. While it is true that the three patients who showed no increase were 27 per cent underweight, two patients who showed marked increases also were 39 per cent and 26 per cent, respectively, underweight. It is difficult to correlate this increase with any other factors.

Lecithin, like cholesterol, is found in all living body cells. It increases with the fatty acids in any lipemia, although usually more

TABLE 11—*Alterations in Plasma Free Fat*

Carbo- hydrate	Case	Grams per Kg	Milligrams per 100 C c			
			Fasting	Hours After Ingestion		
				1	2	6
Inulin	Normal 10	1.3	236	269	193	171
	Normal 11	1.5	114		82	88
	Normal 12	1.3	101	83		189
Inulin	13	1.5	273	267	333	276
	14	1.5	—34	29	91	187
Levulose	7	1.6	164	167	134	134
	8	1.6	430		446	477
	1	1.2	—12	35	89	160
	9	1.3	214	201	109	18
	6	1.1	98	417	374	320
Artichokes	1	1.8	137	247		258
	2	1.4	60	163	103	224
	3	0.7	180		214	209
	4	0.9	269	174	267	
	5	1.5	154	215	241	218
	6	1.2	349	406	244	229

slowly, and the increase is of longer duration, according to Bloor. It is synthesized from fat and phosphoric acid, and probably represents one state in fat metabolism. Bloor³⁰ found a relatively large part of the unsaturated fatty acids of the blood in combinations with cholesterol and a smaller portion combined as phospholipoid. The lecithin findings are not complete, but if it contains one saturated and one unsaturated acid, the latter is probably the same as that of the liver, and relation between blood and liver lecithin must exist. This work tends to support the belief that lecithin represents a means of transportation for fat.

Increases in plasma lecithin occurred in eleven of these experiments. The maximal increase occurred five times in the sixth hour and four times in the second. The average maximal increase was 26.3 per cent.

Increases in free fat were found in thirteen experiments. If the levulose experiments with Case 1 are omitted from the average, the average maximal increase in free fat was 155 per cent. The alterations in plasma fat occurred more promptly and were greater in degree than those observed with the other lipoids.

The interpretation of these puzzling results must await further study and the criticism of other workers. Striking increases in plasma lipoids were observed in the three normal subjects who received only inulin. Patient 11, whose blood sugar fell to 0.05 per cent at the end of six hours, showed an increase in plasma lecithin and cholesterol, but a decrease in fatty acids and free fat. This suggests that the withdrawal of fat from the blood may stimulate the formation of lecithin and cholesterol.

Levulose and artichokes produced, in the diabetic subjects, a lipoidemia characterized by a tendency toward an earlier and more marked increase in plasma fat and fatty acid. The higher the initial

TABLE 12—Average Diet, Insulin and Weight of Diabetic Patients Taking Artichokes

Num ber of Cases	Carbo- hydrate Added as Arti- chokes, Gm	Time, Mo	Diet at Beginning				Diet* at End				Insulin		Weight	
			Car- bohy- drate, Gm	Pro- tein, Gm	Fat, Gm	Calo- ries	Car- bohy- drate, Gm	Pro- tein, Gm	Fat, Gm	Calo- ries	Begin- ning, Units	End, Units	Begin- ning, Kg	End, Kg
17	19	6	51	51	105	1,353	59	53	117	1,501	18	21	36.3	42.4

* Exclusive of artichokes

lipoid values, the less was the tendency for such an increase to occur. In the milder diabetics little or no increase was observed. This may be explained on the ground that, in the latter, the block in the pathway of carbohydrate utilization is but slight whereas, in the more severe cases with more complete block in the pathway, the carbohydrate may be converted in part into fat. In the most severe cases, even this expedient fails. Possibly this explanation may be invoked in a comparison of Patients 9 and 6, who received small amounts of levulose hourly. In the first moderate increases in cholesterol and lecithin with a pronounced fall in plasma fat occurred, while the respiratory quotient was scarcely affected and the urine contained sugar. We may infer that practically no increased combustion of carbohydrate and no conversion into fat occurred. In the second, a marked increase in lipoids, with slight glycosuria and an extraordinary rise in respiratory quotient suggest that conversion of levulose into fat had occurred.

A comparison of the effects of levulose and artichokes on the plasma lipoids may be made in general terms. In Case 1, slightly greater lipoidemia occurred with 71 gm of carbohydrate, as artichokes, than with

48 gm of levulose In Case 6, a decrease in lipoids occurred with 53 gm of carbohydrate in the form of artichokes, as compared with an increase in lipoids when 50 gm of levulose were given in divided doses In general, the slower absorption of carbohydrate with a more prolonged rise in respiratory quotient when artichokes were given caused a less pronounced lipoidemia than did pure levulose

JERUSALEM ARTICHOKE IN THE DIET

The Jerusalem artichoke tuber resembles the potato in appearance, and is easily obtained in large markets It may be prepared for use as food in a variety of ways, such as boiling, baking or frying It must be remembered, however, that if the artichoke is boiled a large part of the inulin will be lost in the water In one experiment, 43 per cent of the inulin was recovered from the water in which the artichokes were boiled Baking is the best method of cooking Mrs Leatherbee, who has served artichokes to many patients in her boarding house for diabetic patients, recommends the following recipe

The artichokes should be washed in cold water and sliced like cucumbers, then baked in a small casserole till soft No water should be added, but mineral oil may be used

The artichokes baked in their skins resemble baked potatoes in appearance but have a very sweet taste Thirty minutes' baking will drive off enough water to cause a loss of 65 per cent in weight Hence, the carbohydrate value must be calculated from their weight before cooking As to the changes that occur in the carbohydrate of the artichokes during baking, we are uncertain The inulin of artichokes is more easily hydrolyzed than that of dahlia tubers During baking, the slow rise of temperature in the center of the artichokes gives opportunity for rapid hydrolysis before the inulase is destroyed The content of levulose or of easily hydrolyzed levulosans may be increased, although we have been unable to demonstrate it At any rate, the baked artichokes are more palatable than the boiled and are eagerly taken, either regularly or at intervals, by the diabetic patients

Twenty-five diabetic patients have taken artichokes in addition to their regular diet (or occasionally in substitution for 5 per cent vegetables) for periods varying from a few days to fifteen months In some cases, the artichokes were taken daily in others, on alternate days, and in one instance, only once a week The average amount of uncooked artichokes taken in a day was 130 gm In Table 12 are given the average results for seventeen patients who took artichokes for at least one month These patients all showed improvement during the test Needless to say, this improvement is not ascribed to the artichokes alone, but the most conservative will agree that they did

no harm In all cases, the patients were able to eat artichokes in these moderate amounts without increasing glycosuria, when present, or producing glycosuria when it was previously absent

Artichokes may occasionally be used to render the urine sugar free without increasing the insulin dose or decreasing the diet Patient 15, a boy, aged 7 years, became sugar free when 100 gm of baked artichokes was substituted for 15 gm of carbohydrate, as orange and Triscuit Patient 16, a woman, took baked artichokes in place of 5 per cent vegetables and fruit for two days Although she thus increased the carbohydrate of her diet by 10 gm, she became sugar free To confirm this result she returned the next day to her previous diet and glycosuria reappeared No inulin or levulose was found in the stools of patients who received moderate amounts of artichokes

The addition of artichokes to the diet apparently makes it possible to add other food too Thus, these seventeen patients increased the total calories of their diets by 11 per cent besides the artichokes, although the insulin dosage was increased by only 3 units This is understandable if one considers the unusual readiness with which levulose favors glycogen formation Helly³¹ has shown that, even in depancreatized dogs, glycogen may be deposited in the liver when levulose is given It may well be that the good effects of artichokes are due to the slow absorption of levulose, increased glycogen formation and consequent improvement of tolerance of the patient and his protection against acidosis

CONCLUSIONS

Clinically, the use of Jerusalem artichokes proved beneficial to a group of diabetic patients who used them for average periods of six months In the amounts given, they were added to the diet without increasing glycosuria or producing it where previously absent, and when substituted for other carbohydrates in the diet, in certain instances, they rendered the urine sugar free During the periods of these tests, patients were able to increase the other components of the diet and to gain weight with only a slight addition to their insulin dosage

Experimentally, the advantages of artichokes seemed to depend on the gradual liberation and slow absorption of levulose, although a small amount of glucose also was present Baked artichokes raised the respiratory quotient in all cases, including cases with acidosis

The increase in the respiratory quotient tended to occur later and to be more prolonged than with levulose

The increase in blood sugar produced by artichokes was definite but less than that produced by an equivalent amount of carbohydrate in the form of levulose

Experiments conducted in order to make comparison with inulin derived from dahlia tubers showed that inulin did not cause a significant rise in blood sugar percentage but that it had a slight though definite effect on the respiratory quotient and metabolism

Increases in the lipoids of the plasma resulted when artichokes and inulin, as well as levulose, were given patients. The greatest increases occurred in the more severe diabetic subjects when levulose was given. In subjects receiving both levulose and artichokes on different occasions, the latter provoked the lesser degree of lipoidemia.

HYPOGLYCEMIA ^{*}

REPORT OF A CASE UNASSOCIATED WITH INSULIN ADMINISTRATION

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The clinical history of a case in which hypoglycemia was associated with symptoms very similar to those occurring in the hypoglycemic shock following overdosage with insulin is here given

REPORT OF CASE

H F C, a Chinese man, aged 33, was admitted to the Peking Union Medical College Hospital, Aug 17, 1924, for gastric analysis and roentgen-ray examination of the gastro-intestinal tract, on account of epigastric distress and feeling of flatulence after meals. The past history was irrelevant, except that he never had any previous attacks in any way resembling that described below, nor any symptoms suggestive of hysteria.

Shortly after admission, the patient developed a diarrhea which lasted for five or six days, three or four stools daily with abdominal cramps. The stools were liquid and fecal, with mucus, but contained no blood. The night before the colon examination, the supper was omitted and he was given 40 c c of castor oil, which produced a number of liquid bowel movements. The next morning, without breakfast, a soapsuds enema was given. Immediately after the enema, the patient had very severe, cramplike pains in the abdomen. He had speech difficulty and was disoriented. The hands and feet were held in spasm resembling the carpopedal spasm of tetany, but no facial phenomenon (Chvostek's sign) was obtainable. The abdominal muscles were rigid. The whole body was in active tremor, the face pale and dripping with profuse cold perspiration, and the pulse weak and rapid, giving a picture of severe shock. During the attack, the patient felt numb and stiff in the face, as well as in the extremities, which could be moved only with difficulty, but there was no blurring of vision, tinnitus or loss of consciousness. The abdominal cramps lasted for an hour, but the spasm of hands and feet did not disappear for two and one-half hours. The picture, clinically, in every way resembled a moderately severe hypoglycemic (insulin) shock, although at the time the possibility of hysteria was also considered.

The colon examination was postponed until two days later when exactly the same preparation was given, in an attempt to reproduce the same syndrome, but without result. During this time, however, the diarrhea had ceased and food in normal quantities had been taken.

^{*}From the chemical laboratory, the department of medicine, Peking Union Medical College

On the assumption that the rigidity of the hands and feet might be due to tetany, blood was taken for calcium and phosphorus determination¹ The results of the blood examination made during the attack and on the second day following are given in the accompanying table

Blood Examination

	Mg per 100 C c Glucose	Mg per 100 C c Calcium	Mg per 100 C c Inorganic Phosphorus	Percentage by Volume Carbon Dioxid Capacity
Aug 25, 1924, without attack	75	11.3	1.7	50.4
Aug 25, 1924, without attack	100		3.8	55.1

The value for calcium was found to be high, while that for phosphorus was very low. The high calcium and normal carbon dioxide capacity, together with the absence of the facial phenomenon, rendered the diagnosis of tetany quite unlikely. Furthermore, the low phosphorus in the blood led one to anticipate a low blood sugar, as the associated presence of these phenomena has been shown² to occur during the process of active insulin action. Banting, Campbell and Fletcher³ have described hypoglycemic shock following insulin injections. When the level of the blood sugar gets as low as 0.075 per cent, patients experience hunger and a feeling of fatigue, accompanied by vasomotor phenomena and tremulous sensations. The blood sugar determined in this case was 0.075 per cent. On account of the hypoglycemia present and the similarity of the symptoms manifested in this case to the hypoglycemic syndrome, one is strongly led to assume that this patient was suffering from a condition analogous to hypoglycemic shock.

We are unable to offer a certain explanation of the etiology of this attack. The writers have not found reports of any similar case in which the blood sugar has been studied. But the facts are that this man had a severe and dehydrating diarrhea, and had been vigorously purged and partially starved before the attack occurred. We think it not unlikely that the depletion of carbohydrate from a susceptible patient by an exhausting diarrhea, fasting and severe purging may sufficiently lower the blood sugar to cause the symptoms of hypoglycemic shock, when given some immediate stimulus (here the manipulations necessary to give an enema) to set them off.

1 The chemical methods employed were (a) Glucose Folin, O, and Wu, H. J. Biol. Chem. **38** 81 (May) 1919. (b) Calcium Kramer, B, and Tisdall, F. F. J. Biol. Chem. **47** 475 (Aug) 1921. (c) Phosphorus Briggs, A. P. J. Biol. Chem. **53** 13 (July) 1922. (d) Carbon dioxide combining power Van Slyke, D. D., and Cullen, G. E. J. Biol. Chem. **30** 289 (June) 1917.

2 Harrop, G. A., Jr., and Benedict, E. M. J. Biol. Chem. **59** 683 (April) 1924.

3 Banting, F. G., Campbell, W. R., and Fletcher, A. A. Brit. M. J. **1** 8 (Jan 6) 1923.

In conclusion, it is desired to call attention to the possibility of hypoglycemic shock, after such a train of events, in a predisposed subject. Before the discovery of insulin, the syndrome of hypoglycemic shock was unknown and unrecognized, and many hysterical attacks, so considered, might prove in reality to be hypoglycemic in nature, if blood sugar determinations were made. In cases of cholera, in which the muscular cramps have usually been ascribed to dehydration, estimations of blood sugar would be interesting, in order to determine whether or not hypoglycemia may play a part in their causation.

Book Reviews

LECTURES ON PATHOLOGY By LUDWIG ASCHOFF, M D, Professor of Pathologic Anatomy, University of Freiburg Delivered in the United States, 1924
Thirty-Five Illustrations

This book contains a number of lectures that were delivered by Professor Aschoff, during his recent visit to the United States. It includes, among others, the Lane Lectures of Leland Stanford University, the Janeway Lectures of the Mount Sinai Hospital, New York, and the Osler Memorial and the Harvey Lectures.

Altogether there are fourteen separate papers. The subjects discussed are varied, they cover a wide range of pathologic data and evidently are not intended to form a consistent series. All, however, are concerned with subjects vital to medicine at the present time. Among these subjects, one finds the following: the reticulo-endothelial system, the pathogenesis of human pulmonary consumption, concept of inflammation, pathologic fatty changes, the normal and pathologic morphology of the suprarenals, atherosclerosis, ovulation and menstruation, the orthology and pathology of the extrahepatic passages, the site of formation of bile pigment, thrombosis, the relation of mucosal erosions to the development of ulcers of the stomach, the goiter problem, especially the goiter of puberty, renal secretions, and renal diseases.

It is impossible, in this review, to give a resume or even to state the main conclusions of the author. On all these problems, he has worked and written for years, and these lectures are really condensations of his numerous papers and monographs. For that reason, many physicians will find this book most useful in obtaining fairly concise statements of Aschoff's voluminous writings.

Of greatest interest, probably, are his discussions of the reticulo-endothelial system and the pathogenesis of human pulmonary consumption. More especially, Aschoff discusses, in connection with the reticulo-endothelial system, the morphology and origin of its elements, and then takes up the reaction of these cells in various pathologic processes, laying stress particularly on their relation to blood destruction, to bile pigment production, to their participation in general metabolic functions, such as the metabolism of fat, the metabolism of proteins, and their relation to the storage and metabolism of iron. Of particular interest, too, is his discussion of this system in relation to defense reactions and ferment production.

Aschoff presents concisely his well known views on pulmonary tuberculosis, emphasizing the importance of recognizing a primary infectious stage, characterized by an exudative, rapidly caseating bronchopneumonia, which is usually single. There may be a reinfection, which is usually multiple, this often occurs at the apex of the lung and results commonly in a process of cicatrization and contraction, this forming the productive type of phthisis.

His concept of inflammation is founded on a broad biologic basis. He analyzes in detail the various factors entering into this process, and presents an interesting but exceedingly complex tabular representation of the inflammatory reactions.

Aschoff emphasizes the complexity of the process of thrombosis and discusses several factors in the formation of thrombi. He takes up so-called spontaneous thrombosis, presenting in a masterly way its morphology and the mechanics of its formation. This chapter is recommended especially to the surgeon for his careful consideration.

Attention is called to the interesting chapter on mucosal erosions. Here appear many statements bearing on the origin and prevention of ulcers of the stomach.

To those interested in the study of goiter, his morphologic discussion of the problem will be of interest. He also draws many comparisons between the disease as it appears in Europe and in this country.

The author has a wide range of detailed information not only in the field of morphology, where he stands supreme, but also in biochemistry, bacteriology, immunity, and especially in comparative pathology. From all these sources, he utilizes and correlates important data in the elucidation of disease processes.

THE PHYSIOLOGY OF EXERCISE. A TEXTBOOK FOR STUDENTS OF PHYSICAL EDUCATION. By JAMES HUFF McCURDY, A.M., M.D., M.P.E., Director of Physical Education Course in the International Young Men's Christian Association College, Springfield, Mass., Editor of the American Physical Education Review. Illustrated. Pp. 242. Philadelphia: Lea and Febiger, 1924.

This book was written to serve as a guide and text for students and teachers of physical education, and most of the material presented was obtained through studies carried on over a period of more than twenty years at the International Young Men's Christian Association College at Springfield. There is an extensive bibliography, and frequent references to the literature on the subject are made. It deals largely with the effects of exercise and the various forms of athletic contests on individuals, both in regard to general constitutional effects and to the influence of exercise on the various organs, with particular emphasis on functional changes.

SYSTOLIC BLOOD PRESSURES IN YOUNG MEN

INCLUDING A SPECIAL STUDY OF THOSE WITH
HYPERTENSION ¹

H S DIEHL, M D

AND

K H SUTHERLAND, M D

MINNEAPOLIS

When considering the subject of hypertension, one is impressed with the indefiniteness of our knowledge. Some of the sequelae of hypertension have long been recognized and life insurance companies have been emphasizing the importance of hypertension, even though unaccompanied by other symptoms. Fahr ¹ estimates that approximately 50,000 persons in the United States die each year from "hypertension heart," and that probably an equal number of deaths reported as apoplexy and Bright's disease are actually the result of hypertension. This would mean that hypertension ranks foremost among the causes of death in this country ². But even with the general realization as to the importance of the condition, we are unable as yet to draw a sharp line between normal blood pressure and low grade hypertension, and we lack definite information concerning the age at which hypertension makes its first appearance.

Alvarez,³ in an attempt to determine what constitutes normal blood pressure in young persons, has made some very excellent studies of the blood pressure records of freshmen students at the University of California. The readings were made over a period of four years and include records of 6,000 men and 8,934 women. He found that 45 per cent of the men had systolic pressures exceeding 130 mm., and 22 per cent had pressures exceeding 140 mm.; that about 12 per cent of the women had pressures exceeding 130 mm. and about 2 per cent had pressures exceeding 140 mm. He found that the average pressure for both men and

¹ From the Student's Health Service and Department of Preventive Medicine and Public Health, University of Minnesota.

² This study was carried out with the aid of a grant from the Research Fund of the University of Minnesota.

1 Fahr, G. E. Hypertension Heart, J. A. M. A. **80** 981 (April 7) 1923.

2 Mortality Statistics, U. S. Census Bureau, Washington, Government Printing Office, 1922.

3 Alvarez, W. C., Wulzen, R., and Mahoney, L. J. Blood Pressures in Fifteen Thousand University Freshmen, Arch. Int. Med. **32** 17 (July 15) 1923.

women tends to drop during the first years of adult life These figures seemed surprisingly high and so have been compared with the blood pressure readings of the freshmen men examined at the University of Minnesota The examinations of the women students have been made under somewhat different conditions and so will be studied separately

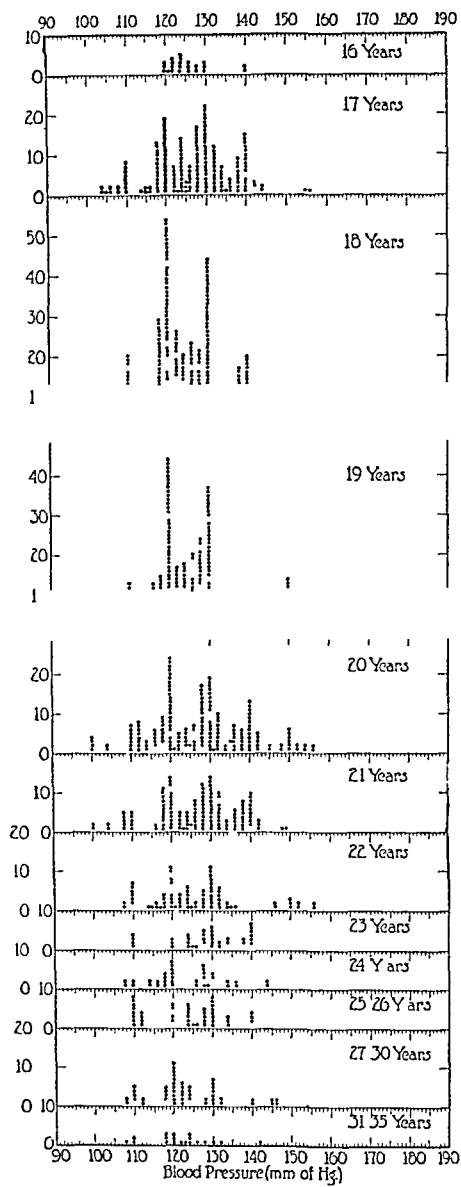


Fig 1—Actual systolic blood pressure readings of 1922 group, each dot representing one case

TECHNIC EMPLOYED

Every student who enters the University of Minnesota is required to have a complete physical examination at the time of his matriculation Blood pressure readings are taken as a matter of routine as part of this examination The auscultatory method is used with mercury manometers or recently standardized aneroid instruments The technic

employed is described by Schneider⁴ in connection with his cardiovascular efficiency test. The student reclines on a bed for several minutes before the blood pressure reading is taken. If on the first reading the systolic pressure is 140 mm or more, the examiner passes on to other

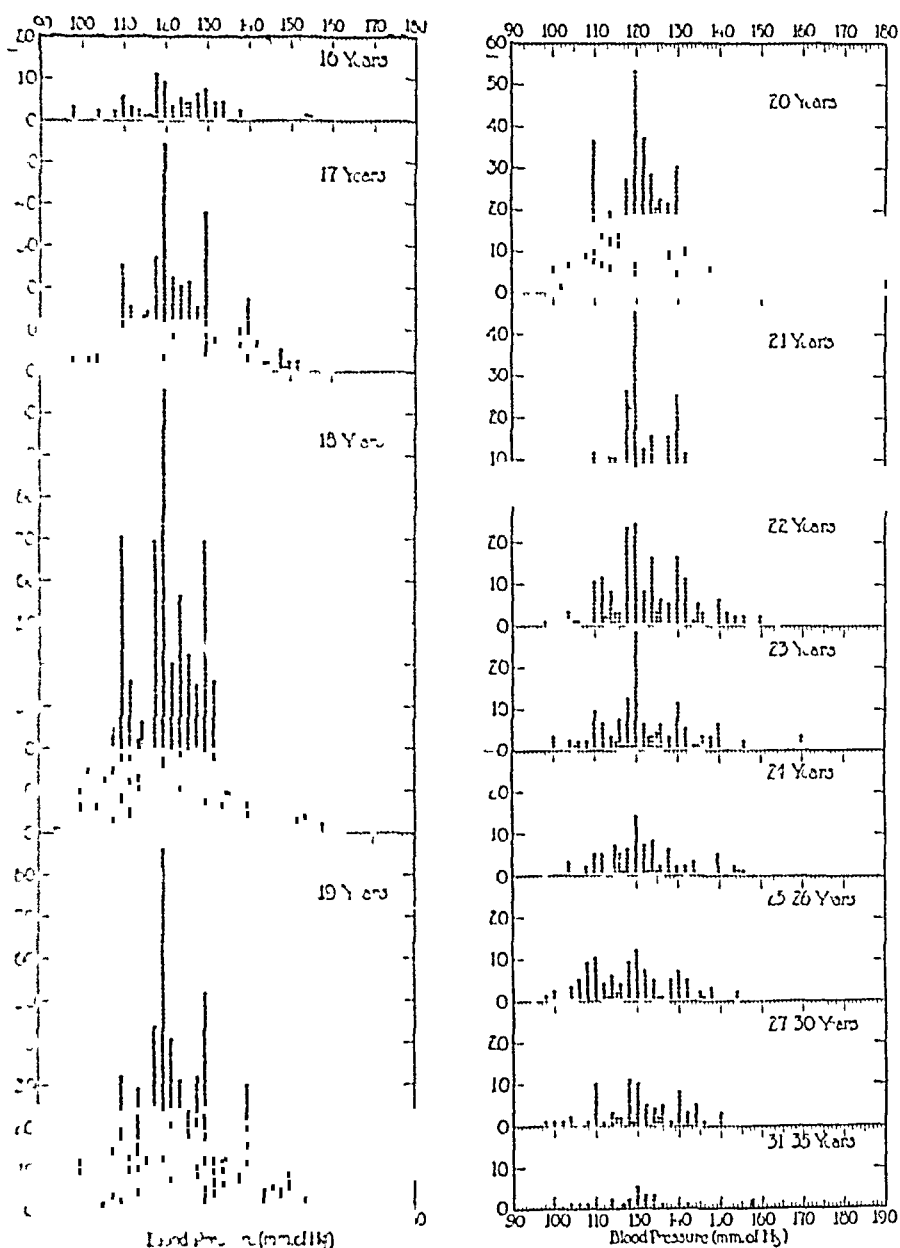


Fig. 2—Actual systolic blood pressure readings of 1923-1924 group, each dot representing one case

students and returns to take a second reading in five or ten minutes. This reading is then recorded. In the 1922 examinations second readings were not made, the original readings being recorded. For this reason the students examined in 1922 are studied as a separate group from those examined in 1923 and 1924.

⁴ Schneider, E. C. Cardiovascular Rating as a Measure of Physical Fatigue and Efficiency, *J. A. M. A.* **74** 1501 (May 29) 1920

TABLE 1—Total Blood Pressures in Men Students According to Age Groups for 1922

Pressure	Ages												Percentages—			
	16	17	18	19	20	21	22	23	24	25-26	27-30	31-35	36-40	Total	Actual	Cumulative
90-94																90-94
95-99																95-99
100-104			8	9	7	5	1	2	1	1	1	1	1	39	2.31	2.31
105-109	3	4	10	11	3	6	3	2	2	1	2	2	1	50	2.96	5.28
110-114	1	10	37	28	18	10	9	5	4	13	7	3	1	145	8.65	13.87
115-119	1	17	43	30	18	16	8	4	7	2	8	3	1	157	9.31	23.19
120-124	13	43	103	80	37	26	22	8	8	15	22	8	1	386	22.89	46.08
125-129	5	27	51	49	26	22	8	7	9	8	5	2	2	219	12.98	59.07
130-134	3	41	70	55	33	28	20	11	7	12	11	2	2	295	17.49	76.57
135-139	1	14	37	21	17	16	3	3	5	5	3	1		121	7.23	83.74
140-144	4	21	31	25	20	16	2	9	5	5	3	1	1	142	8.43	92.17
145-149		2	10	7	5	3	2	2	2	1	4			37	2.19	94.36
150-154		3	9	17	10	3	5	1	2	1	2			53	3.14	97.51
155-159	1	4	5	3	3		2	1		1				20	1.17	98.69
160-164		3	1	3			1	1		1	1			10	0.58	99.28
165-169								1						2	0.12	99.40
170-174	1	2	1		1									4	0.24	99.64
175-179				4										1	0.06	99.70
180-184														4	0.24	99.94
185-189																
190-194																
195-199																
200-204			1											1	0.06	100.00
Total	33	196	417	343	198	151	86	56	50	62	69	19	6	1,086		
Percentage over 130 mm	30.3	45.9	39.5	39.6	44.9	43.7	40.6	50.0	38.0	35.4	34.7	15.7	50.0	40.9		
Percentage over 140 mm	18.1	16.8	13.8	17.4	19.6	14.5	13.9	25.0	14.0	14.5	11.4	5.2	16.6	16.2		
Mode (actual)	124	130	120	120	120	120	130	140	120	110	120	118	130	120		
Mode calculated	117.8	127.6	121.8	123.4	124.4	122.0	119.0	127.0	120.8	122.0	115.4	119.2	120.2	124.2		
Mean	127.1	128.4	126.6	127.3	127.1	126.5	126.5	128.5	126.2	125.0	125.3	121.9	123.8	126.9		
Median	124	128	125	126	128	123	124	128	123	124	122	121	125	126		
Standard deviation	13.4	12.1	11.9	13.4	12.8	11.1	12.3	13.3	11.1	11.9	11.5	7.9	12.8	12.4		
Coefficient of variation	9.4	10.6	10.6	9.1	9.9	11.3	10.2	9.6	11.3	10.5	10.9	15.4	9.6	10.2		
Measure of skewness	0.69	0.99	0.40	0.29	0.21	0.40	0.60	0.11	0.48	0.25	0.86	0.34	0.28	0.21		
Probable error	9.04	8.16	8.03	9.04	8.63	7.48	8.30	8.97	7.49	8.03	7.76	5.33	8.63	8.36		
Unreliability (standard deviation) of the mean	2.35	0.85	0.58	0.72	0.90	0.90	1.21	1.78	1.56	1.50	1.38	1.79	5.12	0.30		
Probable error of the mean	1.59	0.57	0.39	0.49	0.61	0.61	0.82	1.20	1.05	1.01	0.93	1.21	3.45	0.20		

TABLE 2.—Total Blood Pressures in Men Students According to Age Groups for 1923-1924

Pressure	Age												Percentages—	
	16	17	18	19	20	21	22	23	24	25-26	27-29	30-34	Actual	Cumulative
90-94		1	1	1	1				1	2	1		0.11	0.11
95-99	3	4	9	5	2	1	2	6	3	6	4		0.87	0.98
100-104	3	14	15	24	21	16	31	5	2	14	4		4.34	5.34
105-109	2	19	44	29	13	31	31	18	11	21	15	3	4.26	9.71
110-114	11	35	120	77	74	71	31	23	19	24	16	2	13.88	23.57
115-119	15	69	117	87	62	44	70	23	20	15	13	3	13.90	37.48
120-124	17	135	238	170	122	75	52	69	30	24	19	3	24.70	62.48
125-129	14	44	90	72	65	70	44	33	9	7	8		10.68	73.16
130-134	15	53	117	84	52	39	38	17	7	12	16	2	12.86	86.03
135-139	2	24	50	25	19	16	9	8	1	6	5		4.88	90.92
140-144	1	26	22	18	22	14	11	7	7	3		1	4.89	95.78
145-149		8	13	13	13	6	3	3	2	1		2	1.71	97.49
150-154	2	13	13	15	6	7	3	1	2	1			1.57	99.06
155-159	1	1	5	4	4	1	1	1	1				0.42	99.21
160-164		1	1	2	4	1							0.34	99.85
165-169											1		0.03	99.88
170-174			1		2			1			1		0.11	100.00
175-179														
180-184														
185-189														
Total	87	407	875	652	464	283	192	146	95	112	88	8	3,436	
Percentage over 120 mm	25.2	28.9	26.7	28.8	24.5	28.9	28.1	28.0	21.0	20.5	26.1	18.5	26.4	
Percentage over 140 mm	5.7	10.0	7.4	11.0	9.2	9.5	8.8	10.9	12.6	14	6.8	11.1	9.0	
Mode (actual)	118	120	120	120	120	120	120	120	120	120	118	120	120	
Mode (calculated)	115.4	120.4	116.4	120.0	117.8	113.2	114.8	119.4	114.8	116.0	117.0	114.0	120.6	
Mean	122.3	122.8	121.8	123.0	124.1	123.4	122.6	123.3	122.6	119.0	121.5	115.5	122.7	
Median	120	122	120	122	122	120	120	122	120	118	120	116	122	
Standard deviation	11.4	11.4	11.5	11.6	11.3	10.8	10.4	12.2	11.1	11.0	10.6	11.3	11.3	
Coefficient of variation	10.7	10.7	10.5	10.6	10.9	11.4	10.8	10.1	11.0	10.8	11.4	10.7	10.8	
Measure of skewness	0.60	0.21	0.47	0.25	0.55	0.94	0.75	0.82	0.70	0.27	0.42	0.29	0.18	
Probable error	7.69	7.69	7.76	7.82	7.62	7.28	7.01	8.22	7.49	7.42	7.15	7.62	7.62	
Unreliability (standard deviation) of the mean	1.22	0.56	0.39	0.45	0.52	0.64	0.74	1.00	1.14	1.03	1.12	2.17	1.57	0.19
Probable error of the mean	0.82	0.38	0.26	0.30	0.35	0.43	0.50	0.67	0.77	0.69	0.76	1.46	1.06	0.13

STATISTICAL ANALYSIS

The blood pressure records were tabulated and analyzed according to various statistical methods to test their reliability and to show tendencies in the groups. In the 1922 group, there were 1,686 students and in the 1923-1924 groups, 3,436 students, making a total of 5,122 men in the entire series.

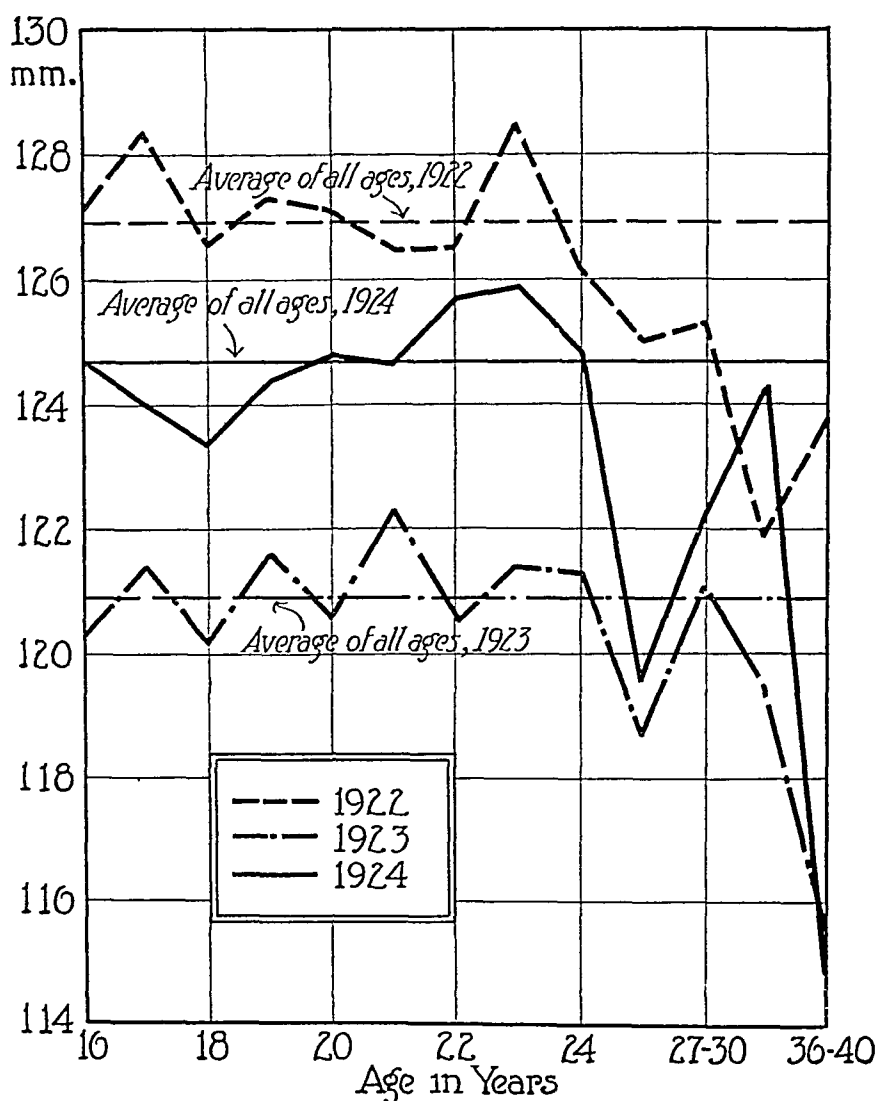


Fig 3—Average blood pressure of each age group and of the total in each of the three calendar years, 1922, 1923 and 1924

Figure 1 is the tabulation of the actual blood pressure readings according to age for the 1922 group. Figure 2 is a similar tabulation for the 1923-1924 group. These charts show the great tendency of examiners to read to even numbers and particularly to tens. In these 5 000 examinations there were almost no pressures recorded as 119 or 121, and less recorded as 125 than as 124 and 126.

TABULATION OF TOTALS

The total number of students whose blood pressures were used in this study was 5 122. Of these, 1,686 entered the University in 1922, 1 826 in 1923 and 1,610 in 1924. Because of the slight differences in technic already described, the 1922 group has been tabulated separately from the 1923 and 1924 groups. In his studies Alvarez has arranged the blood pressures in such groups as 100-104, 105-109, etc.³ Tables 1 and 2 show our readings arranged in the same manner. As can be seen even from these tables, the chief grouping of pressures is between 110 and 130. In the 1922 group, the four students 19 years of age with pressure between 180 and 184 strike as unusual. It seems probable however, that the occurrence of all these in one age group is a coincidence. One had hyperthyroidism, one aortic regurgitation, one transient hypertension and one persistent hypertension.

AVERAGES FOR VARIOUS YEARS

Figure 3 shows the average pressure of each age group for each of the three years, 1922, 1923 and 1924. These curves are quite regular until the groups of 25 years and above are reached. In these higher ages the averages become lower but the groups are so small that these figures probably are not reliable. The curve for 1922 is persistently the highest and the one for 1923 persistently the lowest. The higher pressures recorded in 1922 may be due to the difference in technic, but there is no such explanation for the height of the 1924 curve. This seems to be some evidence of the tendency noted by Alvarez for blood pressures to vary somewhat in different calendar years, a point that merits further study.

MEASURES OF CENTRAL TENDENCY

The arithmetical mean, in other words the average pressure, is shown graphically in Figure 3 and has been discussed in the preceding paragraph. The mean for the entire 1922 group is 126.9 mm. and for the 1923-1924 group 122.7 mm.

The calculation of the unreliability (variation) of the mean, which Alvarez calls the standard deviation of the mean, was made according to Rugg's formula.⁵

The most probable error of the mean then was calculated from this standard deviation.⁶ In practically all the age groups this most probable error is very low.

⁵ $\sigma_m = \frac{\sigma \text{ deviation}}{\sqrt{N}}$, that is, the standard deviation divided by the square

of the number of cases. Rugg, H. O. *Statistical Methods Applied to Education*, New York, Houghton Mifflin Company, 1917, p. 227.

⁶ The most probable error equals the standard deviation multiplied by 0.6745.

The actual (observed) mode is the pressure occurring most frequently in any group. For the different age groups in the 1922 examinations the mode varies considerably, but it is 120 mm for most ages (Fig 1). In the more dependable 1923-1924 examinations, the mode varies only between 118 and 120 mm, 120 mm being by far the most common (Fig 2).

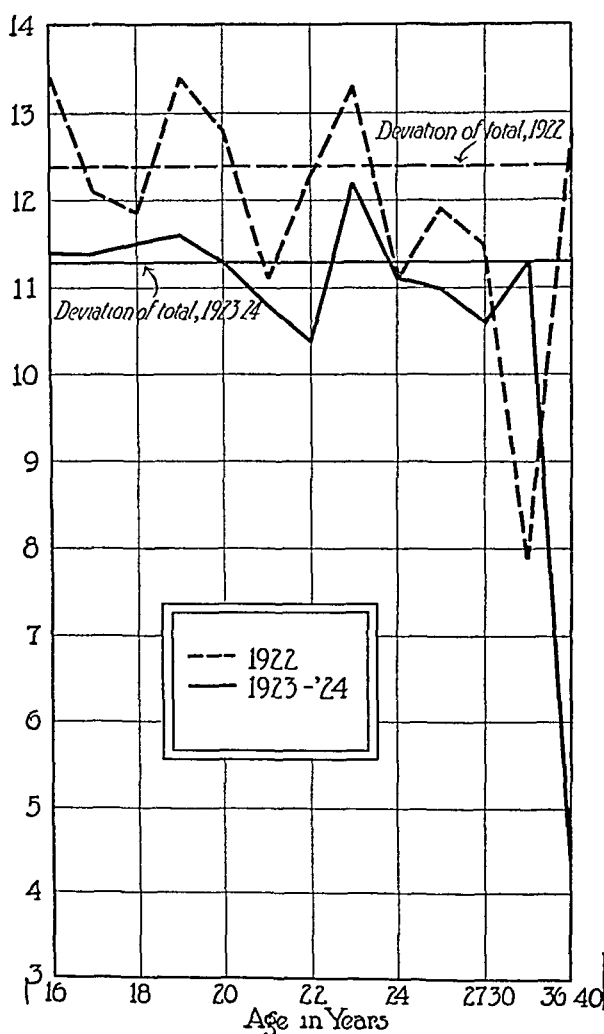


Fig 4—Standard deviation, as given in Table 1, for each age group

The calculated modes have been computed according to Pearson's method⁷. These calculated modes for each age group are lower in all years than the observed modes.

The median pressure is the middle figure, that is, the pressure that has just as many readings above it as below it. In 1922 the median varied between 121 and 128, with the median of the total 126. In the 1923-1924 group, the variation was between 116 and 122, with the median of the total 122.

⁷ The calculated mode equals the mean — 3 (mean — median)

MEASURES OF VARIABILITY

Some ideas as to the reliability of statistical data can be obtained by computing the standard deviation, the coefficient of variation, and the most probable error ⁸

The variability of the standard deviation and the coefficient of variation in the different age groups is shown in Tables 1 and 2 and Figures 4 and 5. The smallness of these measures and the regularity of the graphs shows that statistically the figures are reliable except in the higher age groups, in these the totals are too small to be of value.

SKEWNESS OF THE CURVES

Any curves drawn through the high points of the individual readings as shown in Figures 1 and 2, would be extremely irregular and very confusing. Furthermore, the small differences in pressure that would make such curves irregular are of so little clinical significance that the curves would be misleading. A better idea can be obtained from percentage frequency diagrams shown in Figures 6, 7, 8 and 9. In preparing these charts, groups of ten were used because most physicians think of blood pressures as being "around 120 mm, around 130 mm, around 140 mm" etc., and the readings most frequently recorded, i. e., 110 mm, 120 mm, 130 mm, 140 mm, etc., were used as the midpoints of the groups. The figures in each group were then reduced to percentages and plotted.

These groups were preferred to the ones given by Alvarez because, if one uses groups such as 120-124, 130-134, etc., with high points, such as 120 and 130, at one extreme (Figs. 1 and 2), and then assumes that the data are evenly distributed within the groups, the conclusions will not be absolutely accurate. If groups of five are preferred, they should be from 118 to 122, from 123 to 127, etc.

In the 1922 examinations, the percentage of pressures between 115 and 124 mm is highest in six of the age groups, and the percentage between 125 and 134 mm is highest in seven of the groups. Of the

⁸ The standard deviation was calculated according to the formula

$$S.D. = \sqrt{\frac{\sum d^2}{N} - (\Lambda_T - \Lambda_1)^2}$$

\sum = Number of cases having each pressure

d = Deviation of the pressure from the median of the group

N = Total number of cases in the group

Λ_T = True average

Λ_1 = Even average used in calculating

The coefficient of variation equals the mean divided by the standard deviation, and the most probable error equals the standard deviation multiplied by 0.6745

entire class, 34 per cent had pressures between 115 and 124 mm and 31 per cent between 125 and 134 mm. In the 1923-1924 group, however, the percentage with pressures between 115 and 124 mm is highest for all ages and the percentage between 125 and 134 mm is second in all but two of the age groups. The distributions for the various ages in 1923-1924 are very similar. All of them, of course, are skewed toward the high pressures, the lowest pressure recorded being 90 mm. 30 mm

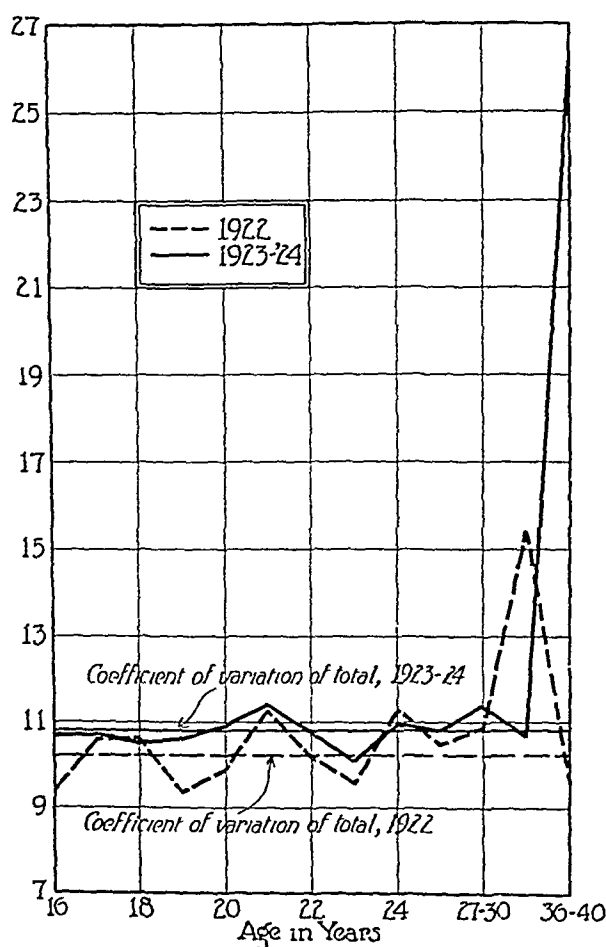


Fig 5—Coefficient of variation, as given in Table 1, for each age group

below the mode, while the upper limits are from two to three times as far removed from the mode.

The measure of skewness was calculated according to Pearson's method⁹. This measure is higher for most ages than is recorded by Alvarez, but with the lower modes in our series, our curves naturally would be more skewed.

⁹ The measure of skewness is obtained by dividing the difference between the mean and the mode by the standard deviation.

SUMMATION CURVES

The summation curves (Fig 10, 11 and 12) have been plotted from the percentages shown in Figures 6, 7, 8 and 9. The first point on each curve was obtained by taking the percentage of pressures between 85 and 94, the second point by adding to this figure the percentage between 95

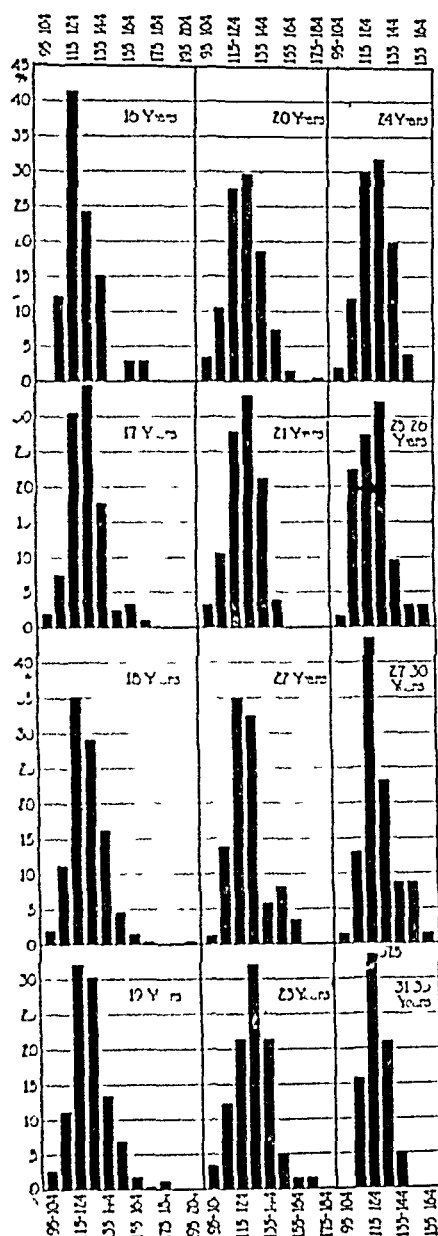


Fig 6—Percentage frequency of various pressure groups of 10 mm for each age in 1922

and 104, the third point by adding to the sum of the first two the percentage between 105 and 114, and so on. Consequently, the numbers to the left of any perpendicular line represent the percentage of cases below the pressure represented by that line, while the numbers to the right of the perpendicular line represent the percentage of cases

above that pressure. These curves were drawn through the points thus obtained without smoothing. Fifty per cent on such a graph represents the median. The skewness of the curves toward the higher pressures is clearly shown. These figures also show at a glance the very slight variation in the pressure curves of the different age groups, and the small percentages with high pressures.

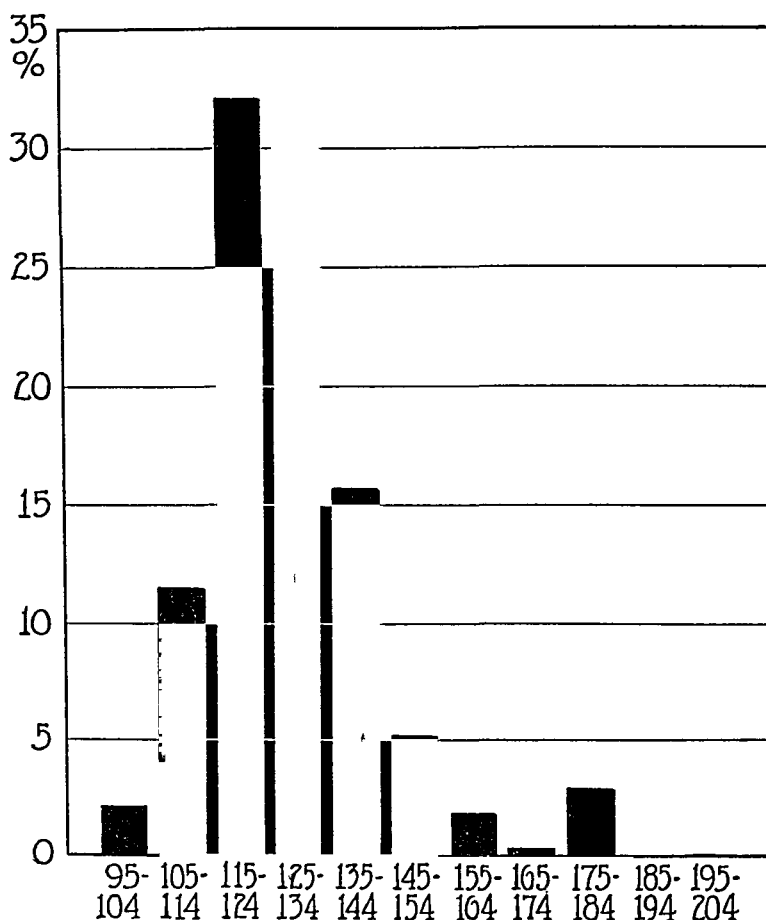


Fig 7—Percentage frequency of various pressure groups of 10 mm for total 1922 group

PERCENTAGE OF HYPERTENSION

It is generally believed that a persistent systolic blood pressure of 140 mm or more is abnormal in young persons, and many feel that even 130 is beyond the upper limit of normality. In the 1922 group, 16.2 per cent had pressures of 140 or more, and 40.9 per cent pressures of 130 or more. In the more carefully examined 1923-1924 group, only 9 per cent had pressures over 140 and 26.4 per cent over 130. These percentages are considerably lower than the ones given by Alvarez, viz 22 per cent exceeding 140 and 45 per cent exceeding 130. In our 1922 group, 1.3 per cent had pressures of 160 mm or more and 0.6 per cent,

of 170 mm or more, in the 1923-1924 group only 0.5 per cent had pressures of 160 mm or more and 0.1 per cent of 170 mm or more. The incidence of hypertension, as shown in Figure 13, varies but little for the different age groups and there is no general tendency, as Alvarez found, for the pressures to be higher in the younger age groups.

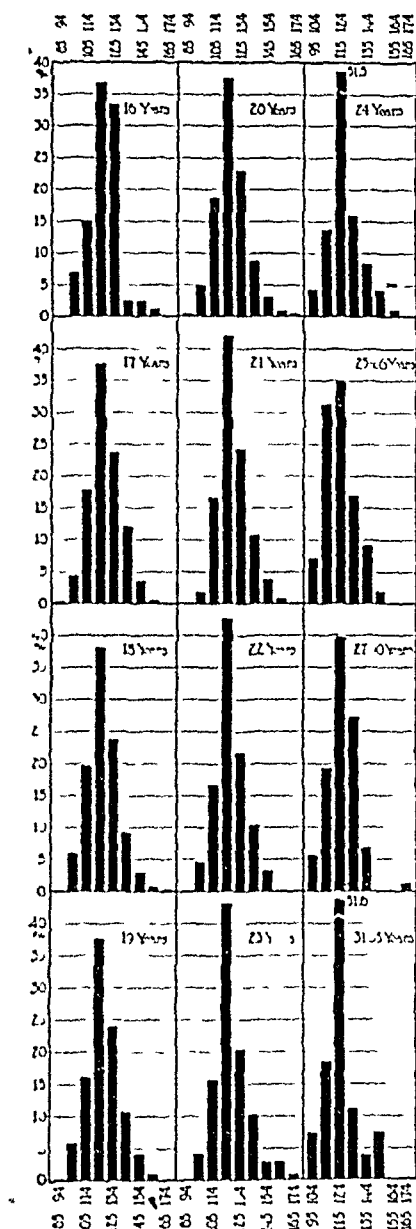


Fig 8—Percentage frequency of various pressure groups of 10 mm for each age in 1923-1924

REEXAMINATION OF STUDENTS WITH HYPERTENSION

After noting the rather high incidence of hypertension as recorded on the 1922 and 1923 examinations, an attempt was made to obtain some information as to the significance of these findings. With this in mind each student whose systolic pressure was recorded as being 140 mm or

more was requested to report for reexamination. No compulsion was used to bring these students back, and no attempt was made to check on those who had left the university. Out of a total of 389 who were recorded as having this degree of hypertension, 209, or 53.7 per cent, were reexamined. Some were reexamined only once, others two, three or four times, the average number of reexaminations being 1.7.

TABLE 3—*Summary of Reexamination of Students Who on First Examination Had Systolic Pressures of 140 Mm or More*

Age	Total Reexamined		Secondary Hypertension		Primary Hypertension							
					Transient		Intermittent		Persistent		Total	
	No	Per Cent *	No	Per Cent *	No	Per Cent *	No	Per Cent *	No	Per Cent *	No	Per Cent *
16	8	18.6	3	7.0	3	7.0	1	2.3	1	2.3	5	11.6
17	29	13.0	3	1.3	15	6.8	6	2.6	5	2.3	26	11.7
18	53	11.5	1	0.2	30	6.5	13	2.8	9	1.9	52	11.2
19	43	11.7	5	1.4	23	6.2	12	3.3	3	0.8	38	10.3
20	15	6.4	2	0.9	6	2.6	5	2.1	2	0.9	13	5.6
21	18	11.5	0	0.0	7	4.5	5	3.2	6	3.8	18	11.5
22	10	9.2	0	0.0	4	3.7	3	2.8	3	2.8	10	9.2
23	9	11.8	2	2.6	4	5.3	3	4.0	0	0.0	7	9.3
24	7	11.9	0	0.0	4	6.8	1	1.7	2	3.4	7	11.9
25-26	10	13.5	3	4.1	5	6.8	2	2.7	0	0.0	7	9.5
27-30	5	7.5	2	3.0	2	3.0	1	1.5	0	0.0	3	4.5
31-35	1	5.0	0	0.0	0	0.0	1	5.0	0	0.0	1	5.0
36-40	1	15.6	1	15.6	0	0.0	0	0.0	0	0.0	0	0.0
Total	209	11.0	22	1.2	103	5.4	53	2.8	31	1.6	187	9.8

* The percentage of the age group as a whole has been calculated by assuming that the findings in the 180 with hypertension who were not reexamined would correspond to the findings of the 209 reexamined and recorded in this table.

RECLASSIFICATION

On the basis of the results of the reexaminations, the students were divided into two main groups, primary hypertension and secondary hypertension. The primary hypertension was further divided into transient hypertension, those with systolic pressures under 130 mm on all but the first examination, intermittent hypertension, those with the majority of readings over 130 mm, and persistent hypertension, those with all readings 140 mm or more. Table 3 shows the age distribution and the calculated percentage of students in each of these groups. This calculation of percentage is made on the assumption that the 180 students with systolic pressures of 140 mm or more who were not reexamined would have shown the same occurrence of secondary transient, intermittent and persistent hypertension as the 209 who were reexamined. The series, of course, is too small to justify fully such an assumption but it is made in order to get some idea as to the proportions of these various groups in the student body as a whole.

SECONDARY HYPERTENSION

Whenever the physical examination revealed any condition that might cause an increased blood pressure, the case was considered as one of secondary hypertension. In Table 4 these various conditions are shown

Hypertthyroidism was diagnosed four times among this group. Heart lesions were considered as a cause of hypertension only when there was a defect of the aortic valve. These cases were easily recognized because of the accompanying low diastolic pressure. The diagnosis of nephritis was based on the persistent occurrence of albumin and casts in the urine, a decreased ability to secrete concentrated urine as shown by the Mosenthal test and, in some cases, an increase of the blood metabolites. It is, of course, possible that in some of these students the urinary findings

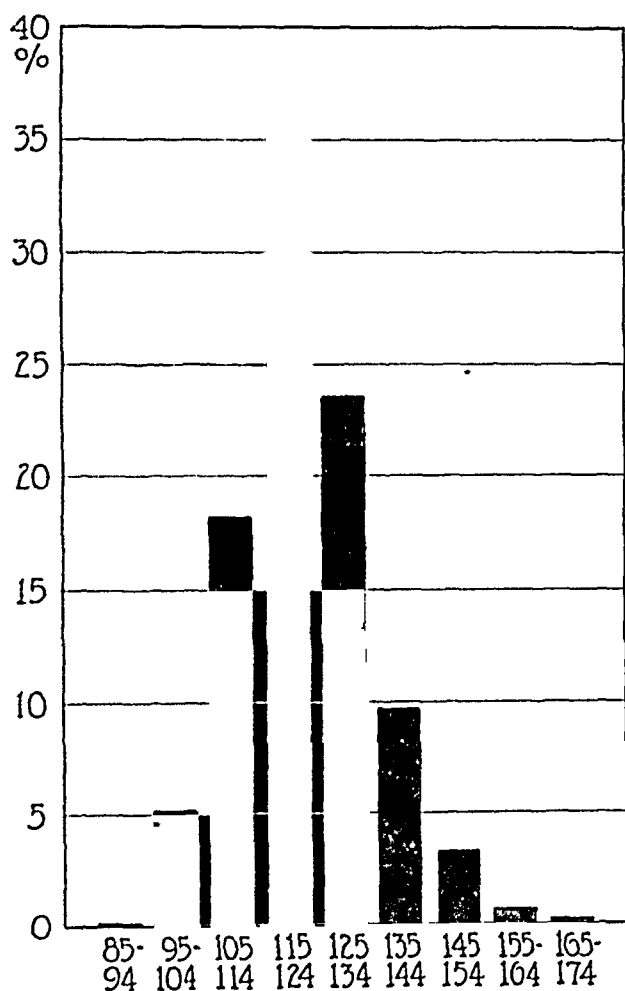


Fig. 9—Percentage frequency of various pressure groups of 10 mm for total 1923-1924 group

were caused by an arteriosclerosis in the kidneys, the cases actually belonging in the group of primary hypertension.

Ten students in the group reexamined showed albuminuria with no other evidences of nephritis. Eight of these had transient, one intermittent and one persistent hypertension. Since hypertension and this type of albuminuria may be evidences of the same general condition, these ten students were considered as having primary, rather than secondary hypertension. The calculated proportion of the 3,538 students who showed a secondary hypertension was —1.2 per cent.

PRIMARY HYPERTENSION

When no condition could be found which would account for an increased blood pressure, the case was considered as one of primary hypertension. A calculated total of 98 per cent of the entire number examined in 1922 and 1923 showed such primary hypertension. The

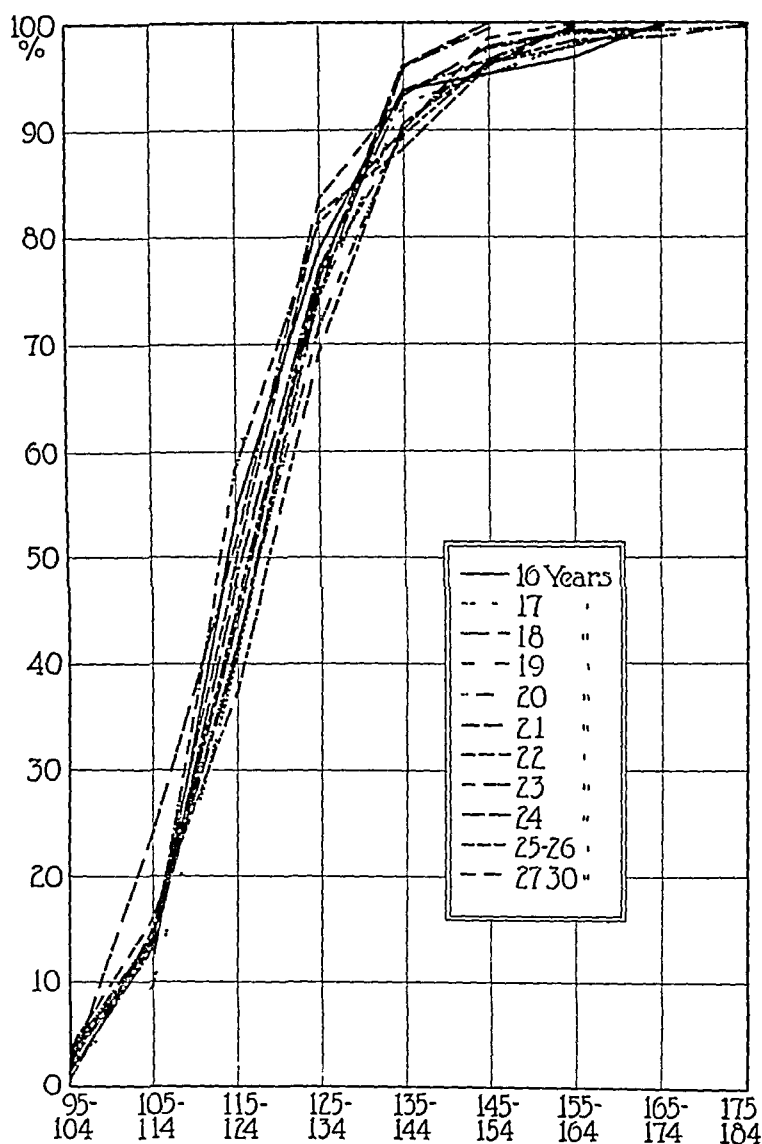


Fig 10—Percentage summation curves for each age group in 1922

occurrence of this condition among the various age groups is surprisingly uniform, except for the 20 year old group, in which it is only 5.6 per cent. The groups of students above 25 years of age are too small for the percentages to be of much significance.

On reexamination the cases with primary hypertension were further subdivided into transient, intermittent and persistent hypertension.

TRANSIENT HYPERTENSION

When a student's systolic blood pressure was recorded on the first examination as 140 mm or more but on subsequent examination was always found to be below 130 mm, his condition was considered as one of transient hypertension. In these students the increased blood pressure

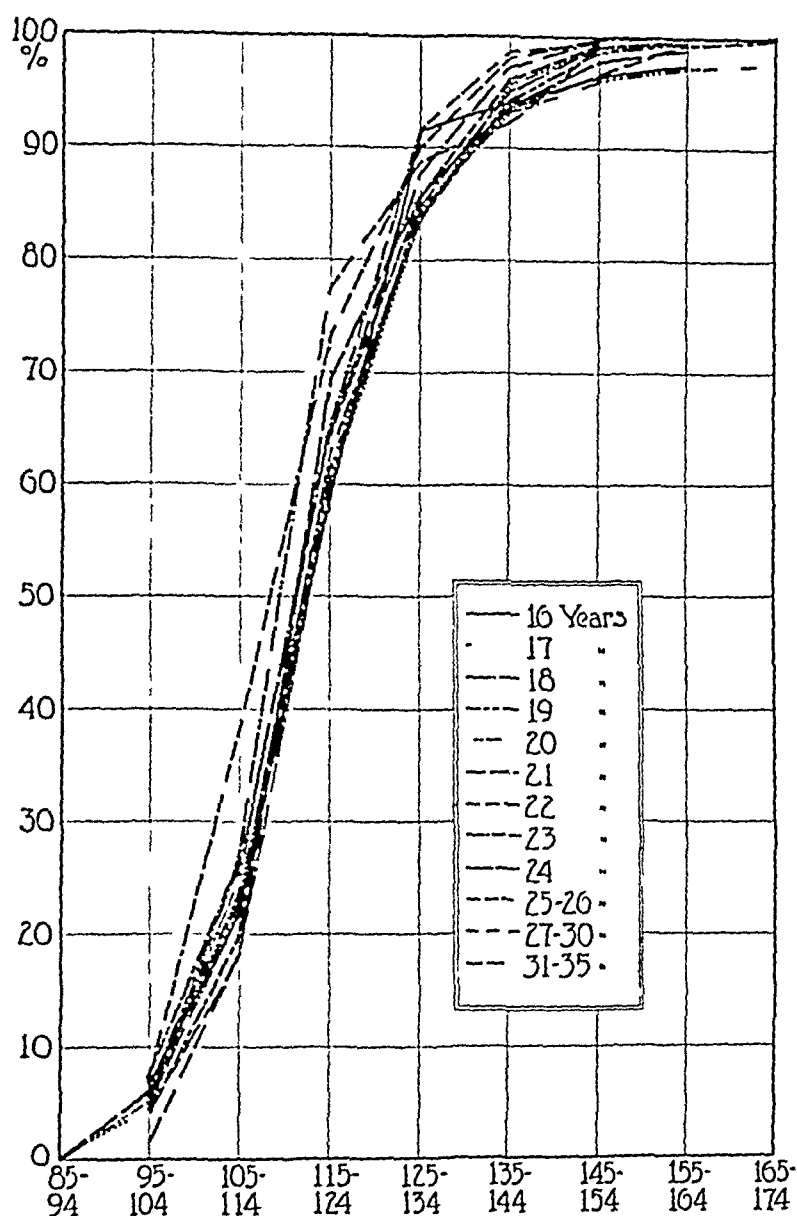


Fig 11—Percentage summation curves for each age group in 1923-1924

probably was caused chiefly by the excitement and hurry of university registration, consequently, it probably has little if any significance. A calculated total of 54 per cent of the entire class had transient hypertension. The distribution by age groups shows the condition most frequent in students of 16, 17, 24 and 25-26 years.

One would expect more excitement over entering college among students of 16 and 17 years than among students a few years older. It also may be surmised that students who enter college at the age of 24 years or more tend to comprise a group more emotionally unstable than would be found in a cross-section of the population of the same age group. Some of these older students may have been teaching school or doing other work for years in order to save sufficient money to go to college. To them this event, of course, would be accompanied by a considerable amount of exhilaration. Others, after leaving high school, may have been unsuccessful in business and so turn to college with the hope that their deficiencies may be overcome. The number of these older students, however, is too small to be significant. The low percentage of transient hypertension in the 20 year old group is striking but no explanation is apparent.

TABLE 4—*Causes of Secondary Hypertensions*

	Transient Hypertension	Intermittent Hypertension	Persistent Hypertension	Total
Hyperthyroidism	0	0	4	4
Heart lesion	1	2	6	9
Chronic nephritis	0	4	4	8
Chorea	0	1	0	1
Total	1	7	14	22

INTERMITTENT HYPERTENSION

When the original blood pressure record was 140 mm or more and the majority of subsequent readings over 130, the case was called one of intermittent hypertension. The calculated total in this group was 28 per cent. The distribution, according to the various age groups, is quite uniform. It is impossible to say what the significance of this condition is, but it seems that when a young person frequently has high blood pressure, he must be at least a prospect for permanent hypertension later in life.

PERSISTENT HYPERTENSION

When the blood pressure was 140 or more on every reading, the condition was called persistent hypertension. The calculated total of students who fell into this group was 16 per cent of the entire class. The occurrence of this condition in the different age groups is somewhat irregular, but the groups are too small to be significant. What the future holds in store for these young persons with persistent hypertension, one cannot say definitely, but the group is worthy of particular study from many standpoints especially as to familial tendencies with relation to hypertension and as to the further course of these individuals in relation to their systolic hypertension and the development of hyperpiesis, essen-

tial hypertension and cardiovascular diseases Fisher¹⁰ of the Northwestern Mutual Life Insurance Company reports that the death rate among 743 persons between the ages of 16 and 29 who were rejected for life insurance because of systolic blood pressures of from 10 to 50 mm or more over the average for the age was 195.9 per cent of the expected rate, that is, the actual death rate was almost double the expected death rate. Among persons of higher ages, the ratio was still greater.

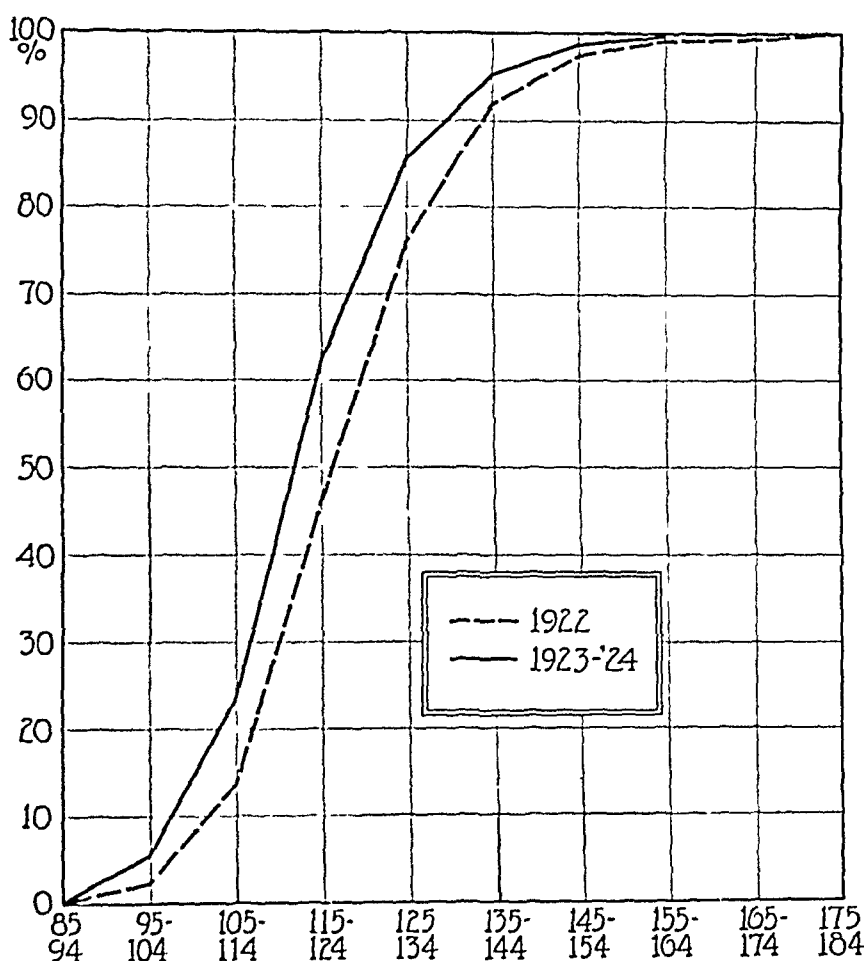


Fig. 12—Percentage summation curves for entire 1922 and 1923-1924 groups

WEIGHT AND HYPERTENSION

The tendency for hypertension to be most frequent in overweight people has been pointed out by life insurance companies and is now rather common information.¹¹ With this in mind, a preliminary study of the weights of the students who were reexamined for hypertension

¹⁰ Fisher, J. W. Report of Blood Pressure Committee, Proc. A. Life Ins. M. Directors of America 9:59, 1922-1923.

¹¹ Hunter, Arthur. Weight and Blood Pressure, Proc. A. Life Ins. M. Directors of America 7:199, 1920-1921.

was made. This did not include a thorough statistical analysis of the data. The results are given in Table 5. On this table no relationship between weight and transient or intermittent hypertension is apparent. In the small group that showed persistent hypertension, however, the most frequent percentage to occur, i. e., the mode, was 108 per cent of normal, the average, i. e., the mean, was 106.6, and the median was 103. Only 3.4 per cent were below 90 per cent of normal weight, while 24.1 per cent were over 110 per cent of normal weight for age and height. This would suggest that even in young people there may be some relationship between weight and persistent hypertension.

TABLE 5—*Weight of Students with Hypertension**

Section	No. of Cases	Mode (Actual)	Mean	Median	Under 90 per Cent of Normal Weight	Over 110 per Cent of Normal Weight
A Transient hypertension	91	100	98.2	100	16.4%	12.0%
B Intermittent hypertension	51	92, 108	100.3	100	13.7%	17.6%
C Persistent hypertension	29	108	106.6	103	3.4%	24.1%
Total average	171	108	103.3	100	13.4%	15.8%

* The figures in the first four columns of this table represent the percentage that the weight is of normal. Calculations were made according to age and height with the use of the table of the Prudential Life Insurance Company.

VITAL CAPACITY AND HYPERTENSION

Table 6 displays the vital capacity readings, expressed in percentages of normal,¹² of the students in the various hypertension groups. In general the vital capacity seems high, but no relationship is evident between the type of hypertension and the percentage of vital capacity.

COMMENT

Although the etiology of hypertension is not yet understood, various contributory factors in its production, such as age, weight, etc., have from time to time been pointed out. Symonds,¹³ in a statistical study of the blood pressures of 150,419 persons accepted for life insurance, found a difference of about 14 mm. between the systolic pressures of persons of light build and persons of heavy build, and about 11 mm. difference between the youngest and oldest age groups. Bell and Hartzell¹⁴ emphasize the importance of body weight over age and show that the age increase in the average heart weight is due to increased body weight and the inclusion of cases of hyperpiesis. From this they inferred that

12 Myers, J. A. Studies on the Respiratory Organs in Health and Disease, *Am. Rev. Tuberc.* 7: 161 (May) 1923.

13 Symonds, B. The Blood Pressure of Healthy Men and Women, *J. A. M. A.* 80: 232 (Jan. 27) 1923.

14 Bell, E. T., and Hartzell, T. B. Studies on Hypertension. The Relation of Age to the Size of the Heart, *J. M. Res.* 44: 473 (June) 1924.

the age increase in blood pressure is due to similar factors Weitz¹⁵ and others have observed the hereditary or familial character of hypertension Alvarez¹ discusses the significance of such possible factors as sex the war, the influenza epidemic, etc And most physicians at one time or another have observed the great tendency of excitement and nervousness to increase blood pressure

TABLE 6—*ital Capacity of Students with Hypertension*

Section	No. of Cases	Mode (Actual)	Mean	Median	Under 90 per Cent of Normal	Over 110 per Cent of Normal
A Transient hypertension	92	91	105.1	104	1.3%	27.1%
B Intermittent hypertension	51	89, 115	103.1	106	11.7%	39.2%
C Persistent hypertension	29	96, 103	100.0	103	17.2%	24.1%
Total average	172	96	103.7	104	8.7%	30.2%

On the basis of the studies reported in this paper, it would seem that there are several factors that may be etiologic in the production of high blood pressures in young persons. On the reexamination of 209 students who on the entrance physical examinations showed systolic pressures of 140 mm or more, 11 per cent were found to have organic diseases, such as hyperthyroidism, aortic regurgitation, chronic nephritis, etc., to which the hypertension observed probably was secondary. Only 15 per cent of the students with pressures of 140 mm or more on first examinations showed persistent elevation of blood pressure. This is the group with real primary hypertension, the cause of which is, as yet, unknown. Twenty-five per cent of the group had intermittent hypertension—a condition that many believe will develop in later life into a persistent hypertension. The other 49 per cent had merely transient hypertension. These transitory increases of systolic blood pressure are responsible for about half the high blood pressures observed among university freshmen. It is probably true that certain individuals are more susceptible to rises of blood pressure than others, but the immediate cause in most cases is excitement or nervous tension. In our studies it was noted that blood pressures were lower when precautions were taken to eliminate excitement. Shepard and Diehl¹⁶ pointed out that hypertension is more frequent among students from small communities than among those from larger cities. In studies on the need for mental hygiene among college students, Morrison and Diehl¹⁷ observed that among students of a psychoneurotic nature the percentage

15 Weitz, W. Zur Aetologie der genuinen odervascularen Hypertension, *Ztschr f klin Med* 96 151 (Jan 27) 1923

16 Shepard, W P, and Diehl, H S. Rural and Urban Health, *J A M A* 83 1117 (Oct 11) 1924

17 Morrison, A W, and Diehl, H S. Mental Hygiene Needs of Freshmen, *J A M A* 83 1666 (Nov 22) 1924

of hypertension was twice as large as among the student body as a whole. The high incidence of hypertension noted by Alvarez among 16 and 17 year old students probably was due to the greater excitement that the younger boys and girls experience on entering college. But the greatest evidence of the influence of excitement is the fact that about

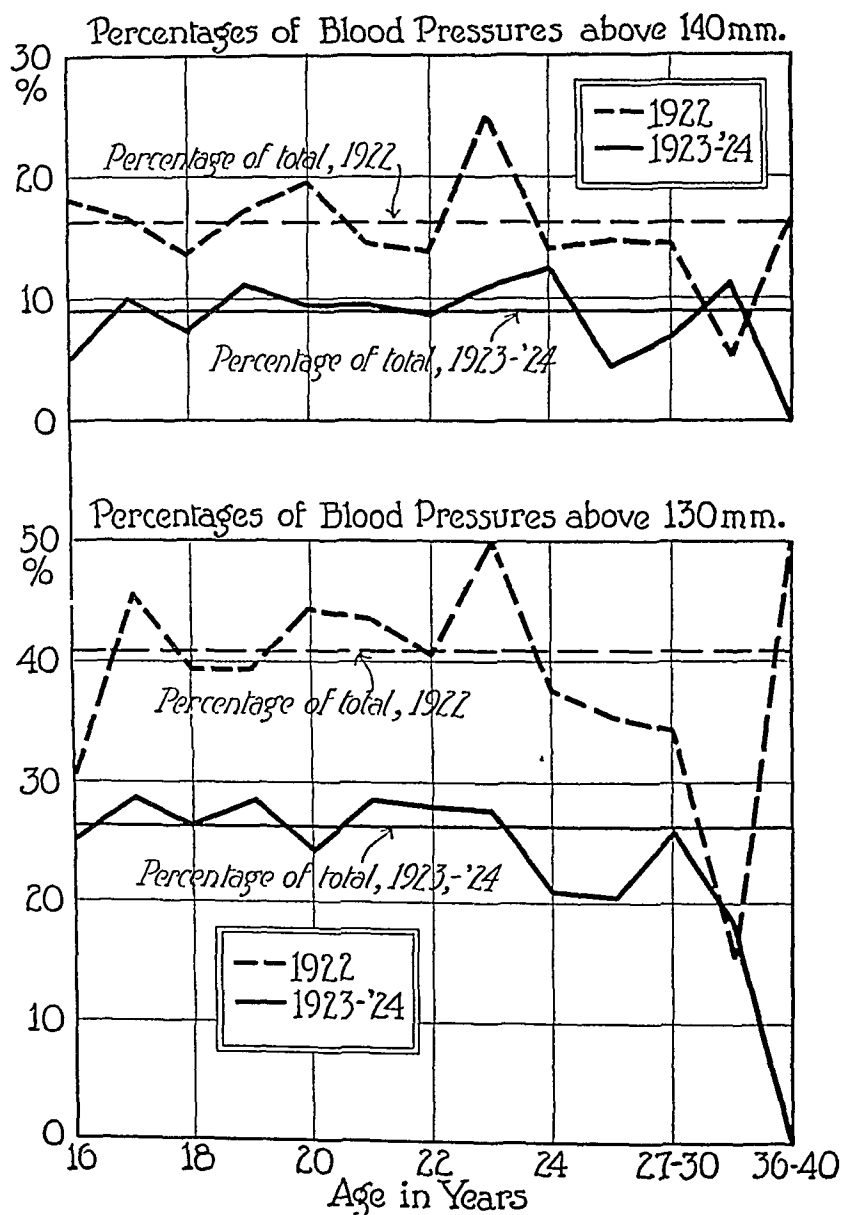


Fig 13—Percentages of blood pressures over 130 mm and percentages over 140 mm for each group in 1922 and in 1923-1924

half the students who showed hypertension on the first examination were found to have normal blood pressures on reexamination.

The grouping of blood pressures around 120 mm, as evidenced by a mode of 120, a mean of 122.7, and a median of 122, offers no basis for a

suggestion that we should modify our ideas as to what constitutes normal blood pressures for young persons Symonds¹³ reported that the average (mean) systolic pressure for all builds at 20-24 years was 124.2 mm. Apparently, if the element of excitement can be eliminated, the generally accepted standards for blood pressure are adequate.

This group of apparently normal healthy young adults who already have shown persistent hypertension offers an unusual opportunity to observe the effect of hypertension on subsequent health and accomplishment. Every possible effort will be made to follow the group both in college and in later life.

CONCLUSIONS

1 A study has been made of the systolic pressures of the 5,122 young men who entered the University of Minnesota in the years 1922, 1923 and 1924.

2 Greater precautions to eliminate the influence of excitement were taken in 1923 and 1924 than in 1922, with the result that the blood pressures in these years were lower than in 1922.

3 In 1922, 16.2 per cent had systolic pressures of 140 or more, in 1923 and 1924 only 9 per cent showed this degree of hypertension. In the latter group, only 0.5 per cent had systolic pressures of 160 mm or more and 0.1 per cent of 170 mm or more.

4 Two hundred and nine students out of 389 who in the 1922 and 1923 entrance examinations showed systolic pressures of 140 mm or more, were reexamined. Only 15 per cent of these proved to have persistent hypertension.

5 The calculated proportion of the entire group examined who showed secondary hypertension was 1.2 per cent, transient hypertension 5.4 per cent, intermittent hypertension 2.8 per cent, and persistent hypertension, 1.6 per cent.

6 Nervousness and excitement seem to be most important factors in the production of transient hypertension in young persons.

7 There seems to be some relationship in this group between overweight and persistent hypertension but none between vital capacity and hypertension.

PATHOGENICITY OF *TRICHOMONAS* *INTESTINALIS**

HIROMU TSUCHIYA, M D

BATTLE CREEK, MICH

The question of pathogenicity of *Trichomonas intestinalis* has long been debated without an apparent solution. The consensus of opinion among parasitologists at present seems to be in favor of nonpathogenicity of the flagellate. In fact, the majority of textbooks treat the infection as of minor importance. Barlow¹ states that the increase of trichomonas is the result and not the cause of the diarrhea. Manson and Bahr² refer to its presence as accidental and not due to a pathologic condition. Minchin³ claims trichomonas to be entozoic in the human intestine and a harmless scavenger rather than a parasite. However, Rhamy and Metts⁴ cite the incidence of local epidemic and endemic occurrence, and all the cases that came under their observation can be frequently traced to the same causes as acute and chronic diarrhea.

Lynch⁵ emphasizes that it is a definitely pathogenic organism, and produces a mild enteritis manifested by intermittent diarrhea. Freund⁶ believes that in some cases of enteritis characterized by diarrhea, abdominal distress and anemia *Trichomonas hominis* is a direct etiologic factor. A marked improvement or a complete recovery is obtained by its removal or a reduction in the number of the flagellates. Smithies⁷ reports twenty-one cases of trichomoniasis, most of which are suggestive of a pathologic condition attributable to this infection, in his enterologic clinic. The catabolic products contained in a large number of these parasites may have a considerable influence as gastro-intestinal irritants, as one of the causes of metabolic changes in the digestive glands, and as a source of the clinical symptom complex of so-called auto-intoxication. Escomel,⁸ strong in his belief of its definite pathology, considers its presence is liable to induce in the liver hepatic abscess similar to that caused by *Entameba dysenteriae*. The infection usually starts as chronic

* From the Clinical Laboratories of the Battle Creek Sanitarium

1 Barlow, N. New Orleans M & S J **69** 299 (Oct) 1916

2 Manson and Bahr. Tropical Diseases, Ed 7, New York, William Wood & Co., p 769

3 Minchin. An Introduction to the Study of Protozoa, London, Edward Arnold, 1912

4 Rhamy, B. W., and Metts, F. A. *Trichomonas*, J A M A **66** 1190 (April 15) 1916

5 Lynch, K. M. Cultivation of *Trichomonas* and Question of Differentiation of Flagellates, J A M A **79** 1130 (Sept 30) 1922

6 Freund, H. A. Arch Int Med **1** 28 (Jan) 1908

7 Smithies, F. Am J M Sc **156** 173 (Aug) 1918

8 Escomel. Lima, Sarmati y Ca., 1919, Bull Soc Path exot, Paris **7** 657, 1914

with intermittent acute attacks, and often induces ulceration, beginning at the rectum and decreasing in intensity upward. At necropsy, Boyd⁹ found pseudomembraneous colitis extending from the ileocecal valve to the rectum. This seems to substantiate the findings made by Escomel.

Musgrave¹⁰ brings out the fact that the pathogenicity of trichomonas in a diarrheal condition is generally accepted by experienced clinicians in the tropics, and credits it with being a cause of dysentery among children. He admits, however, that there can be only slight importance attached to it when present in small number in otherwise healthy persons and that it is pathologic only when present in large numbers or associated with other parasites. Hudley¹¹ experimented with turkeys and found the flagellates in and beneath the mucosa and submucosa of the ceca, and concluded that they probably entered through the goblet cells in the glands of Lieberkuhn, forced in by the peristaltic movement of the intestine in a similar manner to that in which trypanosomes enter the body. In reptile and batrachia, according to Plummer,¹² trichomonas are often present in the circulation, and their presence is associated with a definite and recognizable lesion of the intestinal wall.

Haughwout¹³ attributes its pathogenicity to the production of growth-inhibiting as well as toxic substances, to liberation of injurious metabolic products, to mechanical irritation of the mucous membrane when present in large number, to interference with absorption by adhering to the intestinal walls, and to destruction of the tissues. Levy,¹⁴ in his findings in twenty subjects following saline purge, reports that all the cases can be traced to the history of diarrhea attacks at some time. Somewhat conservative views were expressed by Castellani¹⁵ and Kofoid and Swezy¹⁶ who stated that diarrhea in these cases is often dependent on the number of trichomonas present. Kofoid and Swezy¹⁷ further point out pentatrachomonas as a distinct species on account of its ability to engulf erythrocytes and digest them while the trichomonas does not, a point of view opposed to the nonpathogenic theory advanced by Wenyon and O'Connor.¹⁸

9 Boyd, M. F. *J. Parasitology* **4** 168 (June) 1918, *ibid* **5** 132 (March) 1919.

10 Musgrave, W. E. Flagellate Infestations and Infections. Giardiasis, or Lambliasis, Trichomoniasis, Prowazekiasis, Cercomoniasis, Chilomastixiasis and Tetramitiasis, *J. A. M. A.* **79** 2219 (Dec. 30) 1922.

11 Hudley, J. M. *Res.* **36** 79, 1917.

12 Plummer. Presidential Address, Royal Microsc. Soc., London, 1912.

13 Haughwout, F. G. *Philippine J. Sc.* **13** 217 (Sept.) 1918.

14 Levy, M. D. *Am. J. Trop. Med.* **2** 1 (Jan.) 1922.

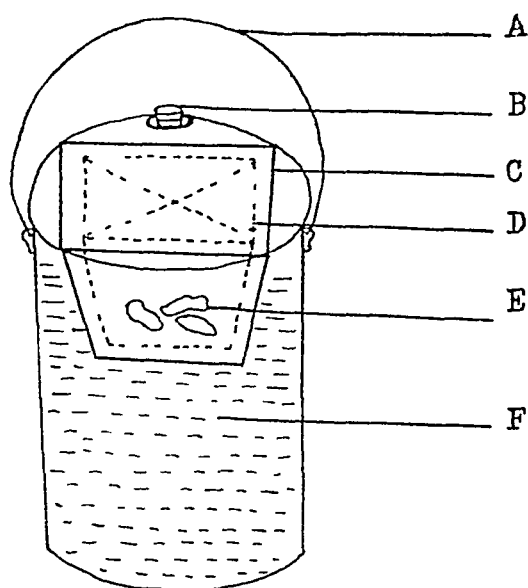
15 Castellani, A. *J. Trop. Med.* **20** 198 (Sept. 1) 1917.

16 Kofoid, C. A., and Swezy, O. *Am. J. Trop. Med.* **1** 41 (Jan.) 1921.

17 Kofoid, C. A., and Swezy, O. *Am. J. Trop. Med.* **4** 33 (Jan.) 1924.

18 Wenyon and O'Connor. *Human Intestinal Protozoa in the Near East*, Wellcome Bureau of Scientific Research, 1917.

During recent months (from July to October), I found considerable frequency in the occurrence of flagellates among the patients at Battle Creek Sanitarium, and this led me to investigate the question not only from the laboratory standpoint but also from a clinical one, hoping that a definite solution might be reached. The tropical zone is the natural climate for *Trichomonas hominis*. According to Barlow, the flagellate infection occurs in 25 per cent of the cases treated, Lynch¹⁹ reported 20 per cent. In this locality, however, 8 per cent should be considered as probably the highest number obtainable, although some of our patients are from southern districts. Barlow,²⁰ however, states that most of the patients that came under his observation were from middle and eastern



Copper bucket, A, handle, B, stopper, C, cover, D, paper pail, E, feces, and F, hot water

states. Using various laboratory findings and clinical data obtained from the department of internal medicine of the sanitarium, I shall endeavor to throw additional light on the subject.

The laboratory method for the detection of *Trichomonas hominis* need not be described fully here, as it has been presented by Hegner and Becker²¹ and Lynch and Hogue²². The method employed in connection with this work was a modification of the one described by them, and is briefly given here.

19 Lynch, K. M. South M. J. **10** 286 (April) 1917

20 Barlow, N. Am. J. Trop. Med. **4** 23, 1924

21 Hegner, L. W., and Becker, E. R. Parasitology **9** 15 (Sept.) 1922

22 Hogue, M. J. Waskia Intestinalis, J. A. M. A. **77** 112 (July 9) 1921

METHOD

Collection of Stool—The stool used in the examination is not allowed to become cool at any time. It should be passed directly into a paper pail (2 quart [2 liter] oyster pail with a metallic clasp), and this pail placed immediately in a warm bucket, as shown in the accompanying illustration. The warm bucket must have been filled with water as hot as the hand can stand but not boiling. The specimen is sent at once to the laboratory for examination. Although Barlow advocates a fresh specimen that has not been subjected to external temperature, by using this method I often find positive cases when the findings are otherwise contrary.

Microscopic Examination—A direct smear is made from the stool, preferably with a little fragment of mucus if present. A drop of physiologic sodium chlorid solution suffices to distribute the smear uniformly under a cover slip. The examination is made, on a warm stage especially provided for this purpose, first by low and then by high power to ascertain the morphology of the organism. It is necessary to examine several smears taken from different parts of the stool before a definite finding is reported. If the case is negative, a pea sized stool should be inoculated into the culture.

Culture—The culture used for this purpose is the ovomucoid medium originated by Hogue. It is made as follows. The whites of six eggs should be beaten thoroughly with an egg beater, gradually mixed into 600 c c of boiling saline solution, and boiled for fifteen minutes. The mixture should be filtered by aid of a suction pump, first through a thin layer of gauze and then through cotton. The filtrate should be placed in 7 c c tubes, each of which should be autoclaved at 15 pounds (6.8 kg.) pressure for twenty minutes. A portion of about pea sized stool should be inoculated and incubated for twenty-four hours at 37 C. The flagellate, if present, is usually found on the surface of the medium, particularly against the sides of the tube. Several smears should be carefully scrutinized before a negative report is given. I found this method superior to the direct microscopic, especially in cases in which there was only slight suspicion of a positive finding. It is often the case that the culture method reveals the motility of flagellates when the direct method fails. The cyst formation of the flagellate is not included in the consideration, as several authors are agreed as to its absence. Although I do not agree with Lynch²³ entirely concerning the possibility of cyst formation, there is no doubt that the trichomonas undergoes a series of transitional stages in its morphology, i. e., from a round, cystlike structure to a fully organized, oval form with a characteristic jerky movement.

²³ Lynch. New York M. J. **101**: 886, 1915.

TABLE 1—Cases in Which Mary

Case	Age	Meat Consumption	Fecal Analysis							Urinary Analysis						Metabolism	
			Mucus	Blood	Gram per Cent	Gram per Cent	B. coli	B. welchii	B. acidophilus	Albumin	Casts	Sugar	Indican	Indol-Acetic Acid	Toxin Test	Carbon Dioxid Tension	Acetone
1	51	Moderate	0	0	35	65	++	+	+	0	0	0	16	+	+	42	0
2	48	Heavy	0	0	20	80	+++	+	0	0	0	0	4	+	+	37	+
3	62	Moderate	0	0	20	80	++	+	0	0	0-1 Hyaline	0	0	+	Trace	36	+
4	64	Heavy	0	0	20	80	+++	—	0	0	0-2 Hyaline	10-80	0	Trace	+	36	Trace
5	27	Little	0	0	35	65	+-	+	+	0	0	0	Trace	+	++	38	0
6	44	None	0	0	40	60	+	+	+	2½	3-4 Hyaline	0	Trace	Trace	Trace	28	+
7	55	None	0	0	20	80	+++	+	0	0	0-1 Hyaline	0	12	+++	++	36	0
8	58	Moderate	0	0	20	80	++	+	0	0	0	0	0	+	Trace	42	0
9	37	Moderate	0	0	20	80	+++	+	0	0	0	0	0	+	Trace	39	Trace
10	32	Little	0	0	35	65	++	+	+	0	0	0	0	0	Trace	44	Trace
11	59	Little	0	0	20	80	+++	+	0	0	1-2 Hyaline	0	0	+	Trace	33	0
12	34	Moderate	0	0	15	85	+++	++	0							36	0
13	41	Little	0	0	15	85	++	++	0	0	0	0	2	+	++	39	0
14	68	Heavy	Much	0	25	75	+++	+	+	0	0-1 Hyaline	0	0	0	0	37	+
15	45	None	Mixed	0	20	80	++	+	0	0	0	0	Trace	0	Trace	42	Trace
16	26	Moderate	0	0	20	80	+++	+	0	0	0	0	0	0	0	40	0
17	41	Heavy	0	0	25	75	---	+	+	0	0	0	2	+	Trace	41	+
18	48	Little	0	0	25	75	---	+	+	0	0	0	5	Trace	Trace	37	0
19	57	Moderate	Some	0	20	80	---	—	0	0	0	0	3	Trace	+	36	0
20	56	Little	0	0	20	80	++	+	0	0	0	0	1	+	+	40	0
Positive per cent			15	0			100	100	35	52+	315+	52+	578+	789+	894+		40
Average					23.5	76.5											37.95

* In this and Table 2, C V R means cardiovascular renal

Blood Analysis			Gastro Intestinal		Circulatory Symptoms				Additional Laboratory Tests	Diagnosis	
Hemo-lobin	Red Cells	White Cells	Symptoms	Proctoscopic Exam	Nervous Symptoms	Blood Pressure					
						Sys-tolic	Dias-tolic	Cardiac	Nutritional Symptoms		
41	4,700,000	10,200	Pain, flatulence, nausea, constipation	Spastic colitis	Headache, irritability, insomnia	114	76	0	None		Neurocirculatory asthenia, intestinal toxemia
42	4,400,000	7,200	Pain, flatulence, constipation	Spastic colitis	Irritability	112	70	0	None		Intestinal toxemia
43	4,400,000	7,500	Pain, flatulence, constipation	Spastic colitis	Headache, irritability, insomnia	134	82	0	Loss of weight	Gastric, 0, 0, 0, 0, 0, 0	Obesity, intestinal stasis
44	4,400,000	4,500	Pain, flatulence, nausea, vomiting, constipation	Rectal stasis	Headache, irritability	100	64	0	Rundown, loss of weight		Diabetes mellitus
45	4,800,000	7,700	Flatulence, nausea, constipation	Colonic and rectal stasis	Headache, irritability	106	60	0	Rundown, loss of weight		Neuropathic
46	4,600,000	7,400	Pain, flatulence, constipation	Rectal stasis	None	175	115	C V R *	Rundown, fever, loss of weight		Cardiovascular renal disease
47	4,850,000	6,000	Constipation	Spastic colitis	None	220	170	C V R	Rundown, loss of weight		Cardiovascular renal disease, cholelithiasis
48	4,800,000	7,700	Flatulence, constipation	Colonic stasis	Irritability	106	68	0	None		Intestinal toxemia
49	4,700,000	7,400	Flatulence, constipation	Spastic colitis	None	110	60	0	None		Intestinal toxemia
50	4,900,000	6,700	Pain, flatulence, nausea, constipation	Spastic colitis	Headache, irritability	118	58	0	Rundown, loss of weight	Gastric, 36, 14, 36, 66, 66, 78	Gastric neurosis
51	4,800,000	5,400	Flatulence, constipation	Rectal stasis	None	192	92	C V R	Rundown, loss of weight		Chronic interstitial nephritis, hemiplegia
52	4,710,000	9,300	Pain, flatulence, constipation	Rectal stasis	Headache, irritability	95	60	0	None	Eosinophils, 1 per cent	Migraine
53	4,870,000	6,600	Pain, flatulence, constipation	Spastic colitis	None	112	72	0	Rundown		Intestinal toxemia
54	4,830,000	6,200	Flatulence, constipation	Colonic stasis	Headache, irritability, insomnia	160	90	0	Rundown, loss of weight		Mucous colitis
55	4,700,000	6,000	Flatulence, constipation	Rectal stasis	None	82	58		Rundown		Mucous colitis, obesity
56	4,730,000	8,700	Flatulence, constipation	Spastic colitis	Headache, irritability	108	62	0	Rundown, loss of weight		Constipation (spastic), retroversion of uterus
57	4,600,000	6,000	Flatulence, constipation	Spastic colitis	Headache, irritability	116	88	0	Rundown		Osteoarthritis, colitis
58	4,600,000	7,200	Flatulence, nausea, vomiting, diarrhea	Mucous colitis	Headache, irritability, insomnia	114	72		Rundown	Gastric, 0, 0, 0, 0, 0, eosinophils, 2.5 per cent	Hypothyroidism, hysteria, diarrhea
59	4,700,000	5,700	Pain, flatulence, nausea, constipation	Spastic colitis	Headache, irritability	116	81	0	Rundown		Constipation (spastic)
60	4,720,000	4,500	Pain, flatulence, constipation	Spastic colitis	Headache, irritability, insomnia	110	70	0	Rundown	Gastric, 18, 0, 14, 18, 34, 0	Cholelithiasis, constipation (spastic)
			Pain	50	Headache	60				Rundown	70
			Flatulence	95	Irritability	70				Fever	5
			Nausea	30	Insomnia	25				Loss of weight	45
			Vomiting	5							
			Constipation	95							
			Diarrhea	5							
61	4,621,500	6,510									

Fixation of Wet Smear—When a positive finding is made, the smear is fixed and stained in order to determine the number of flagellates which differentiate it from *Pentatrichomonas hominis*, present in the species. Hot Schaudinn's solution followed by Heidenhain's iron-hematoxylin is used in the staining.

Morphology of Trichomonas Hominis—This is carefully studied by following the description given by Kofoed and Swezy. Especial attention is directed toward a clear differentiation of the species from *Pentatrichomonas hominis*. For full details on the morphology of the flagellate, the reader may refer to the article by Kofoed and Swezy.

The pathogenicity of *Trichomonas hominis* is centered on gastrointestinal symptoms, particularly diarrhea of varying degrees. In order to make the study conclusive, not only the laboratory findings in feces, urine and blood, as well as the metabolism, but also the symptom complex of gastro-intestinal, cardiac, nervous and nutritional origins have been tabulated. The entire group of the cases has been divided into two classes, one having a large number of *Trichomonas hominis* in an average microscopic field, and the other a few in each. This enables the reader to make a comparative study of the cases as to the severity and complication in the clinical entities. Further, in several instances gastric analysis and differential blood count are added, thus making the clinical and laboratory pictures more comprehensive.

Laboratory findings tabulated here are by no means complete. Only those data necessary for interpretation of the subject have been selected.

FECAL ANALYSIS

Fecal examination is of prime importance in dealing with flagellate infection. It has been proved by experiments carefully conducted by Hegner²⁴ that the putrefactive type of bacterial flora is unfavorable for the growth of intestinal flagellates, while the aciduric type is favorable for their proliferation. His contention on this unique finding is indeed very interesting. Metabolic products of aciduric bacteria provide a medium suitable for *Trichomonas muris*, while indol, skatol and other amino-acid derivatives are injurious to the growth and activities of the flagellates. Thus, with albino rats, he found *Trichomonas muris* the most abundant after the flora had become fermentative by prolonged feeding of the animals with dextrin and a mixed diet. I cannot say at this moment that his experimental deduction is fully in accord with that of Hegner, the full report of which will be published at a later date.

As shown in the accompanying tables, the fecal examination reveals several items in regard to the presence of blood and mucus, the per-

²⁴ Hegner R. W. Am J Hygiene 3 180 (March) 1923

centage of gram-positive and gram-negative bacteria, and the relative proportion of *Bacillus acidophilus*, *Bacillus welchii* and *Bacillus coli*-like organisms expressed by the plus sign

Mucus and blood are seldom found in the cases in which a large number of *Trichomonas hominis* are detected in the stools. The finding is contrary to what Woodcock,²⁵ Lynch, Escomel and Rhamy have brought out. I agree with Levy in making a statement so conservative as "a few instances." Smithies found blood in sixteen cases out of twenty-one, but in none of twenty cases (Table 1) that came under our observation was blood evident either chemically or macroscopically. It tends to prove that *Trichomonas hominis*, differing from *Entamoeba dysenteriae*, is not a hemolytic protozoa, neither has its presence any relation with inflammatory changes brought about in the intestinal wall, if any are present. Diarrhea, so characteristic of the infection, as well as the blood and mucus findings, as indicated by many authors, provide the basis in some part of the present study.

The terminology of "normal stool" as defined by Smithies in his article is not clear to me. However, throughout all our cases there has not been a single stool which could be called "normal" in a bacteriologic sense. The findings, therefore, correlate with those of Smithies and, incidentally, are quite contrary to what Hegner advocated in his experimental study. *B. acidophilus* exhibits neither symbiosis nor antagonism in connection with the presence of *Trichomonas hominis* in the stools. Whatever may be the metabolic products of *B. acidophilus* and of putrefactive bacteria, no appreciable effect is noted on the proliferation of the flagellates. Throughout all the cases, the predominance of coli-like organisms cannot be disputed. Coupled with this, *B. welchii* is present in all, thus proving that indol, skatol and hydrogen sulphid are little hindrance to the growth and multiplication of *Trichomonas hominis*. It is safely stated, therefore, that the type of intestinal flora is independent of the flagellate. Regardless of the flagellates, the flora depends exclusively on the condition of nutrition and the kind of diet given. The putrefactive flora harbors *Trichomonas hominis* as frequently as the fermentative type. The change of flora from aciduric to putrefactive does not exhibit any advantage in decrease of the number of *Trichomonas hominis*, and the determination of the infection can never be induced by feeding the patients a diet favorable for the proliferation of putrefactive bacteria. Such an assumption requires no comment on the therapeutics of the disease.

Contrary to Hegner's belief, the transformation has taken place, though slowly, as *Trichomonas hominis* disappeared from the stools.

25 Woodcock J. Roy Army M. Corps 29 290, 1917

The carbohydrate diet exaggerates the peristalsis of the intestine in such a way as to expel its content very rapidly, thus, the absorptive power of the intestine, particularly of the ascending and transverse portions, becomes more or less retarded, and this affords a chance for the flagellates while actively motile to escape into the stools. In the condition of putrefactive flora, however, the toxic products are quickly absorbed by intestinal mucus, and the feces are formed of of scybalic consistency—the result of complete absorption. During the absorptive process of the intestine, a large number of *Trichomonas hominis* lose their morphologic characteristics, some even undergo structural changes, and doubtless partial or complete dissolution of protoplasm may be brought about. In my experiment with albino rats, at levels further away from the ileocecal valves, the number of *Trichomonas muris* became noticeably diminished. Thus, in the cecum the average number of *Trichomonas muris* in the rats fed with stock diet was eighty-two, at the hepatic flexure, five, while in the descending colon it was three, and in the feces, one. This can be explained by the greater absorptive power of the ascending and the transverse colons and the fact that the cecum fails to function. In the putrefactive condition, the sojourn of the flagellates in the cecum is explained by the favorable alkalinity and also by the lessened onward migration of fecal matter from the cecum to the lower part of the colon.

URINARY ANALYSIS

Urinary examination is not considered as important as fecal, since the infection is primarily of intestinal origin. Only the few data necessary for the interpretation of the flora are tabulated, together with the albumin, sugar and types of casts. Indican, indol, acetic acid and toxin tests serve the purpose in dealing with the bi-products of putrefaction. In looking over the table, irregularity of findings is noted despite the presence of a large number of *Trichomonas hominis* in the stools. The possible relationship of the flagellates to putrefactive bi-products, as mentioned by Hegner, is quite doubtful in view of our clinical observations.

METABOLISM

The metabolism of the subjects showed no appreciable variation in the study. None of the patients presented abnormality in carbon dioxide tension, with the exception of one or two. In these this was attributed to the organic diseases from which the patients were primarily suffering, the flagellate infection had no bearing on the condition. These carbon dioxide tension findings correlate with those made by Barrow. The presence of acetone in expired air in a few instances does not bear out the pathogenicity of flagellate infection.

BLOOD ANALYSIS

Next to the fecal examination, the blood analysis gives an index no less important in the clinical study of trichomoniasis. In most parasitologic diseases, the secondary anemia and eosinophilia are common occurrences. Musgrave and Smithies attribute the low hemoglobin and low red blood count in the infection to the pathogenic effect of *Trichomonas hominis*, while Barrow asserts that the blood picture remains constant, if there are no other diseases present that could account for the abnormality in the hematologic picture. Throughout there was no case in which hemoglobin was higher than 90 per cent in the first series, and 91 per cent in the second, while the lowest were 67 and 63 per cent, respectively. The number of erythrocytes presented a practically normal picture with the two exceptions in which the lowest hemoglobin was quoted. If it is granted that the comparatively low hemoglobin with normal red cell count is of flagellate origin, the clinical picture thus shown is nothing but the result of the average findings obtainable in such a series of cases and cannot be attributed solely to the trichomonas infection. One of the exceptional cases cited above was diagnosed as pernicious anemia. Out of ten cases of trichomoniasis, Freund found two associated with pernicious anemia. The white cell count showed a wide variation, from 5,400 to 10,200. Leukopenia was more prevalent in the whole series than leukocytosis. Although Barrow asserts that leukopenia occurs in any protozoan disease, it cannot be interpreted as a consequence of the trichomonas infection, as these patients came here primarily for treatment for some other chronic disorder of nervous, cardiac or nutritional origin. In such, leukopenia may occur.

DIFFERENTIAL BLOOD COUNT

A differential blood count was made on five cases in which marked eosinophilia was absent. The highest eosinophil count obtainable was 2.5 per cent and the lowest 1 per cent. The test was added in case the blood picture was suggestive of anemia, either primary or secondary. In each instance, there were some irregularities in the morphology of the erythrocytes. From this finding coupled with the blood count and hemoglobin determinations, the cases were diagnosed. Rhamy and Metts report a moderate degree of eosinophilia, from 6 to 12 per cent, in all the cases, while Smithies quotes sixteen cases of eosinophilia out of twenty-one. Barrow states that there were no cases of trichomonas infection that showed a single eosinophil in several examinations of blood stains. Eosinophilia is considered as one of the clinical indexes to most of the parasitic infections, but with trichomoniasis it has no particular bearing.

GASTRIC ANALYSIS

A fractional gastric test also was made in eight cases in which there were three cases of hypochlorhydria, one case of hyperchlorhydria, two cases of achylia and two cases of normal acidity. The tables given here tend to show that hypochlorhydria is more frequently found with trichomoniasis than otherwise. Marked hypochlorhydria was observed by Levy, and eleven cases of achylia and eight cases of hypochlorhydria

TABLE 2—Cases in Which Food

Case	Age	Ment Con- sump- tion	Fecal Analysis							Urinary Analysis						Metabolism	
			Mucus	Blood	Gram + per Cent	Gram — per Cent	B coli	B welchii	B acido- philus	Albu- min	Casts	Sugar	Indi- can	Indol- Acetic Acid	Toxin Test	Carbon Dioxid Tension	Acetone
21	40	Little	0	0	20	80	+++	+	0	0	0	0	0	+	+	36	Trace
22	80	Little	Some	Trace	20	80	+++	+	0	0	0	0	4	0	+	36	0
23	49	Heavy	0	0	25	75	+++	+	+	0	0	0	0	+	Trace	36	0
24	75	Moderate	0	0	25	75	+++	+	+	0	0	0	7	0	+	30	Trace
25	60	Heavy	0	0	40	60	+	+	+	0	0	0	0	+	+	39	Trace
26	22	Heavy	0	0	20	80	+++	+	0	1½	0	0	16	+	+	39	0
27	62	Moderate	0	0	30	70	++	+	+	Trace	3-4 Hyaline	0	3	+	Trace	36	0
28	51	Moderate	0	0	40	60	+	+	+	0	0	24 32	0	+	0	38	Trace
29	58	Heavy	0	0	20	80	+++	+	0	0	2-3 Hyaline	0	Trace	+	+	38	Trace
30	65	Moderate	Little	++	20	80	+++	+	0	0	0	0	3	+	++	30	0
Positive per cent			20	20			100	100	50	20	20	10	60	80	90		50
Average					24	76											35.8

by Smithies. Although our series do not bear out the high abnormal findings of Smithies, there is a tendency toward the predominance of hypochlorhydria, if any. The findings, however, are not conclusive, as such a condition may be attributable to metabolic and nutritional disturbances, from which the majority of our patients suffer and which are, in my opinion, an accidental coincidence in the course of trichomoniasis.

The clinical symptoms tabulated here are the sum total of the present complaints of the patients abstracted from case histories, together with the necessary data obtained through physical examinations by their attending physicians. For the sake of convenience, I classify the symptoms under four headings: gastro-intestinal, nervous, circulatory and nutritional disturbances, the first being the most important and the rest more or less subsidiary to the first.

Trichomona Hominis Here Found

Blood Analysis			Gastro-Intestinal			Circulatory Symptoms				Additional Laboratory Tests	Diagnosis
Hemo- globin	Red Cells	White Cells	Symptoms	Proctoscopic Exam	Nervous Symptoms	Blood Pressure			Nutritional Symptoms		
91	5,000,000	5,100	Flatulence	Rectal stasis	None	95	65		None	Gastric 0, 0, 0, 28, 42, 18	Arthritis, chronic follicular prostatitis
88	4,770,000	6,000	Pain, flatu- lence, nausea, constipation	Spastic mus	None	130	75		Rundown, loss of weight		Arteriosclerosis
88	4,750,000	9,200	Flatulence, constipation	Colonic stasis	Headache	200	110	C V R	None		Obesity, cardiovascular renal disease
87	4,900,000	6,900	None		Irritability	120	70	Chronic myocarditis	None		Rheumatoid arthritis
90	4,800,000	5,700	Pain, flatu- lence, con- stipation	Colonic stasis	Irritability, insomnia	160	90	0	Rundown	Gastric 0, 0, 8, 12, 10, 14	Hypochlorhydria, intes- tinal toxemia
91	4,510,000	7,500	Pain, flatu- lence, con- stipation	Colonic stasis	Headache, irritability, insomnia	110	60	0	Rundown	Gastric, 0, 10, 0, 20, 10, 22	Neurosthenia
89	4,220,000	9,000	Pain, flatu- lence, diarr- hea		Headache, irritability, insomnia	90	50	Tachy- cardia	Rundown, loss of weight	Gastric, 0, 0, 0, 0, 0, 0 eosinophils, 1 per cent	Diarrhea
88	4,000,000	9,600	Pain		None	170	84	Myocar- dial in- sufficiency	None	Eosinophils, 1.5 per cent	Diabetes mellitus, obesity
90	4,940,000	5,100	Flatulence, nausea, con- stipation	Rectal stasis	None	100	60	0	Rundown, loss of weight		Hemorrhoids, urticaria
63	2,610,000	3,900	Flatulence, nausea, vom- iting, con- stipation	Rectal stasis	Irritability	95	50	Mitral regurgi- tation	Rundown, loss of weight	Eosinophils, 2 per cent	Permeous anemia
			Pain	70	Headache	90			Rundown	60	
			Flatulence	50	Irritability	10			Loss of		
			Nausea	70	Insomnia	30			weight	40	
			Vomiting	10							
			Constipation	70							
			Diarrhea	10							
86, 2	4,550,000	6,810									

GASTRO-INTESTINAL SYMPTOMS

Pain—This pain may be either epigastric or colonic. The epigastric pain may be interpreted as of mechanical, chemical and nervous origin, and the colonic as due either to local or functional disturbances. Local pain and tenderness that is not accompanied by a constitutional manifestation has little diagnostic value. Out of twenty cases in Table 1, ten had this symptom; in Table 2, five. In these series, the pain symp-

tom occurred in one-half the cases, seven of which were epigastric in nature. In the majority of the cases, the abdominal pain was distributed in the right iliac fossa, which is suggestive of cecal disorders.

Flatulence—This was a very common occurrence in our cases, especially in those with intestinal putrefaction. The symptom was prevalent in 90 per cent of the first series and in 80 per cent of the second. The flatulence may have a vague significance, but depends largely on the associated disorders, chiefly of gastro-intestinal origin.

Nausea and Vomiting—Nausea was shown in six cases of the first series and in two of the second, and there were only two patients in the whole series who vomited. Nausea and vomiting in these series were brought about by pernicious anemia, diabetes and nervous reflex due to irritation of vagus and phrenic nerves or perhaps to organic diseases of the central nervous system, and are by no means symptoms referable only to trichomoniasis.

Constipation and Diarrhea—The pathogenicity of trichomoniasis has been based chiefly on the production of diarrhea, either acute, chronic or intermittent. This has been emphatically brought out by Woodcock, Rhamy, Freund, Smithies, Lynch, Barrow and Escomel. In their clinical symptomatology, without diarrhea there is nothing to indicate that *Trichomonas hominis* plays a pathogenic rôle. A remarkable contradiction was shown in our series, in which there were only two cases of diarrhea out of thirty cases of trichomoniasis, while constipation occurred in nineteen cases of the first series and in six of the second, and the remainder had neither constipation nor diarrhea in their clinical histories. Moreover, constipation was an outstanding feature in trichomoniasis in our experience. It can be safely stated, therefore, that diarrhea, if present, is due to some other factor of mechanical, chemical or nervous origin. I agree with O'Connor and Wenyon that diarrhea is a more or less accidental happening in the course of a disease due to some other causes. While trichomonas may be associated with a diarrheal condition, it cannot be considered as a cause of diarrhea, this has been clearly proved in our daily examination of stools in which *Trichomonas hominis* is seldom encountered in watery specimens. Some of these cases of diarrhea have been attributed to the excessive fermentative activity of intestinal bacteria, while the consideration of *Giardia* (*Lamblia hominis*) and *Entameba dysenteriae* in this connection also may deserve special mention.

PROCTOSCOPIC EXAMINATION

Proctoscopic examination shows that the majority of the cases of trichomoniasis have been diagnosed as spastic colitis or stasis at some

point in the colon. These findings are further suggestive of constipation, commonly prevalent among the patients.

NERVOUS SYMPTOMS

Nervous symptoms, such as headache, irritability and insomnia, cannot very well be considered as resulting from the trichomonas infection and are either the result of some other pathologic condition or of a neuropathic tendency revealed in the history. These are tabulated merely to present some data necessary for comparison with the studies made by other authors. It is evident that the symptoms mentioned in the foregoing are more referable to intestinal autointoxication than to any other single factor.

NUTRITIONAL SYMPTOMS

General debility, such as loss of weight, a general run down condition and fever are not attributable to the trichomoniasis alone. Any disease related to disturbances in nutrition or of a systemic nature, so common among our patients, manifests similar results.

Nervous and nutritional symptoms, therefore, are not to be considered as conclusive evidence of trichomoniasis, but instead are the common symptoms of many chronic ailments. According to other writers, the important symptom, so far as diagnostic value is concerned, is diarrhea, a cardinal symptom of trichomoniasis. If the positive theory holds true, therefore, the patients should experience a certain degree of diarrhea, either acute, chronic or perhaps intermittent, with constipation, accompanied by a characteristic clinical syndrome. Conclusively, however, constipation is a uniform finding in our series. Nervous and nutritional symptoms are nothing but the outcome of constipation and are not due to trichomoniasis in which they have no significance.

CIRCULATORY SYMPTOMS

The blood pressures shown in the table are not out of the ordinary, considering the age of the patients and the diagnosis given. Hypertension in our series is mostly suggestive of cardiovascular complication, while hypotension indicates nutritional disturbance of some sort.

The amount of meat consumed by the patients before entering the institution was tabulated in order to show the possible effect of high protein on the number of *Trichomonas hominis*. That a high meat diet is productive of intestinal proteolysis needs no special mention. According to Hegner, bi-products of putrefaction, such as indol, skatol, hydrogen sulphid and ammonia, are unfavorable to the existence of *Trichomonas muris* in the large intestine. The higher the extent of putrefaction, the fewer the number of *Trichomonas muris* in the

intestine If his theory can be applied clinically, the low protein consumers are more liable to harbor *Trichomonas hominis* than the high meat eaters Tables 1 and 2, however, does not encourage the hypothesis Irrespective of the diet taken, the flagellates were present in large number

The diagnoses of the cases are tabulated to show the symptom complex exhibited by the patients correlated with the organic or functional disorders rather than with trichomoniasis

Comparative study of trichomoniasis in relation to the number of the flagellates is also made in order to ascertain the possible variation as to its pathogenicity Musgrave and Levy are inclined to believe that the number of *Trichomonas hominis* is directly proportional to the extent of the infection In the average findings tabulated, there is not enough evidence in either series to be conclusive in this regard

CONCLUSION

- 1 *Trichomonas hominis* is not a pathogenic but a harmless flagellate present in the large intestine

- 2 Whether they are present in large or small number, neither laboratory nor clinical pictures bear evidence conclusive enough to warrant pathologic diagnosis

- 3 Diarrhea mentioned by many as characteristic of trichomoniasis, has no clinical aspect in these series, instead constipation is a common occurrence with no diarrheal history in the past Furthermore, proctoscopic diagnosis emphasizes the fact that constipation is a usual accompaniment in these cases

- 4 Nervous symptoms and nutritional disturbances as manifested by these cases are attributable not to the presence of *Trichomonas hominis*, but to some organic or functional disorder or to constipation secondary to such

- 5 The intestinal flora is independent of the presence of *Trichomonas hominis* The type of intestinal flora does not alter the number of the flagellates Hegner's assumption in regard to albino rats is not feasible in its clinical application

PRIMARY VASCULAR NEPHRITIS, OR RENAL PERIARTERITIS NODOSA *

J JAY KEEGAN, MD

OMAHA

A recent case at the University Hospital afforded an unusual opportunity by early nephrectomy and later by necropsy to observe the development of a primary vascular nephritis of infectious origin. The operative specimen presented the characteristic pathologic appearance of periarteritis acuta nodosa and the necropsy specimen, two months later, showed the transformation of this lesion into early arteriosclerosis and chronic vascular nephritis. Associated with this renal vascular pathologic condition was a similar lesion of the cystic artery of the gallbladder, the pancreatic artery and the splenic artery.

REPORT OF CASE

History—Mrs. C., aged 24, entered the University Hospital, Sept. 13, 1924, with the complaint of general malaise of three weeks' duration, weakness and loss of weight, from 118 to 82 pounds (53.5 to 37.2 kg.), over a period of several months. The history was unreliable as the patient was a morphin addict, she had been using 'bromidia' for several weeks and was irrational. She was moaning but not complaining of pain. She looked older than her age and had a gravis pallor. Physical examination revealed marked dental infection. The heart, lungs, abdomen, pelvis and extremities were negative. The temperature was 103, pulse 120, respirations 25. The leukocyte count totaled 20,000, polymorphonuclears 90 per cent. The specific gravity of the urine was 1.020, albumin and sugar tests were negative, there were no casts or red blood cells, a culture of a catheterized specimen on blood agar was negative. A blood culture was negative, the blood sugar was 139 mg., the spinal fluid was normal, blood and spinal Wassermann reactions were negative. The mental condition improved in a few days, the elevation of temperature and polymorphonuclear leukocytosis persisted, but the patient made no complaint and insisted that she felt fine. Roentgenograms of her teeth showed many alveolar abscesses. Distinct tenderness and rigidity were elicited in the right side of the abdomen and in the right kidney region.

Surgical consultation was called, September 26, on account of the persistence of the abdominal symptoms in the right side. There were sepsis and a few pus cells in the urine but no other localizing signs. An exploratory operation was advised, with a preoperative diagnosis of a surgical condition of the right kidney. At operation, September 29, a small anterior right abdominal incision was made and the gallbladder, stomach, appendix and pelvis were explored with negative findings. This incision was closed and the right kidney explored through a right flank incision. The kidney seemed slightly enlarged, the fatty capsule being rather adherent, it was removed. Gross section of this kidney showed numerous white milary nodules all located at the corticomedullary border. These looked like milary tubercles (Fig. 1). Two or three larger white areas with hemorrhagic borders were found in the cortex. The cortex otherwise was rather pale, but with normal markings, averaging 6 mm. in thickness. The capsule stripped easily leaving a smooth surface, except for the few larger slightly elevated nodules noted above.

* From the University of Nebraska College of Medicine.

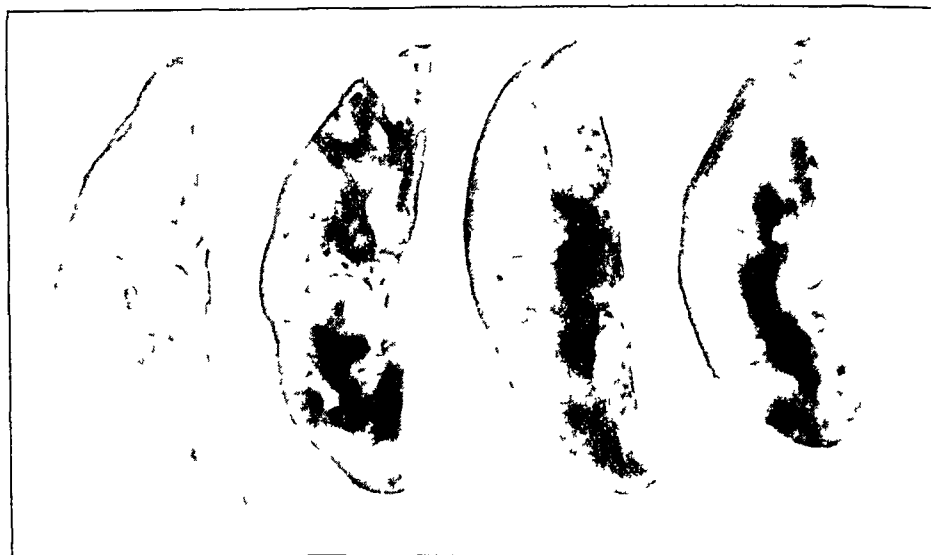


Fig 1—Cut surface of right kidney removed at operation, showing white miliary nodules and hemorrhagic infarct due to swollen and infiltrated arcuate arteries (actual size)

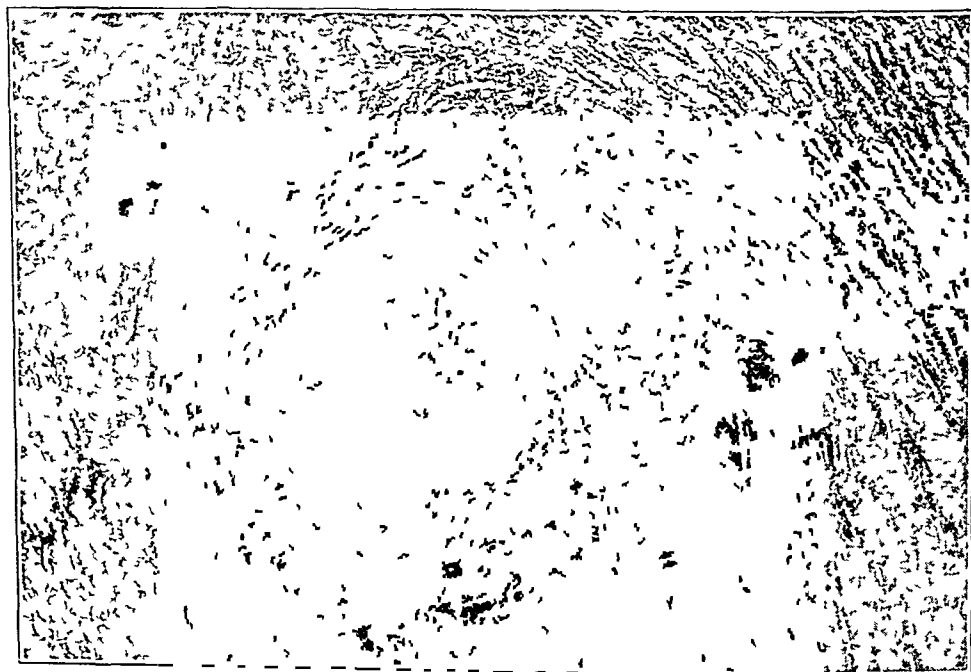


Fig 2—Acute inflammatory lesion of arcuate arteries of right kidney

Microscopic examination of sections of this kidney showed the milary nodules to be markedly swollen and infiltrated arcuate arteries (Fig 2). The inflammatory reaction involved the entire artery wall, intima, media and adventitia. The intima was markedly swollen from edema and infiltrated with numerous mononuclear cells and polymorphonuclear leukocytes. In places there was extensive necrosis of this swollen intima. A small lumen containing a few red blood cells usually persisted. The muscularis in many places was almost indistinguishable on account of the inflammatory cell infiltration, elsewhere it was fairly well defined but with swollen cells. The adventitia was densely infiltrated and markedly thickened with mononuclear cells, a few polymorphonuclear leukocytes, an occasional eosinophil and edema. The process was definitely limited to the artery wall, it did not extend appreciably into the surrounding kidney tissue. Search for bacteria in kidney sections stained by the Giemsa method

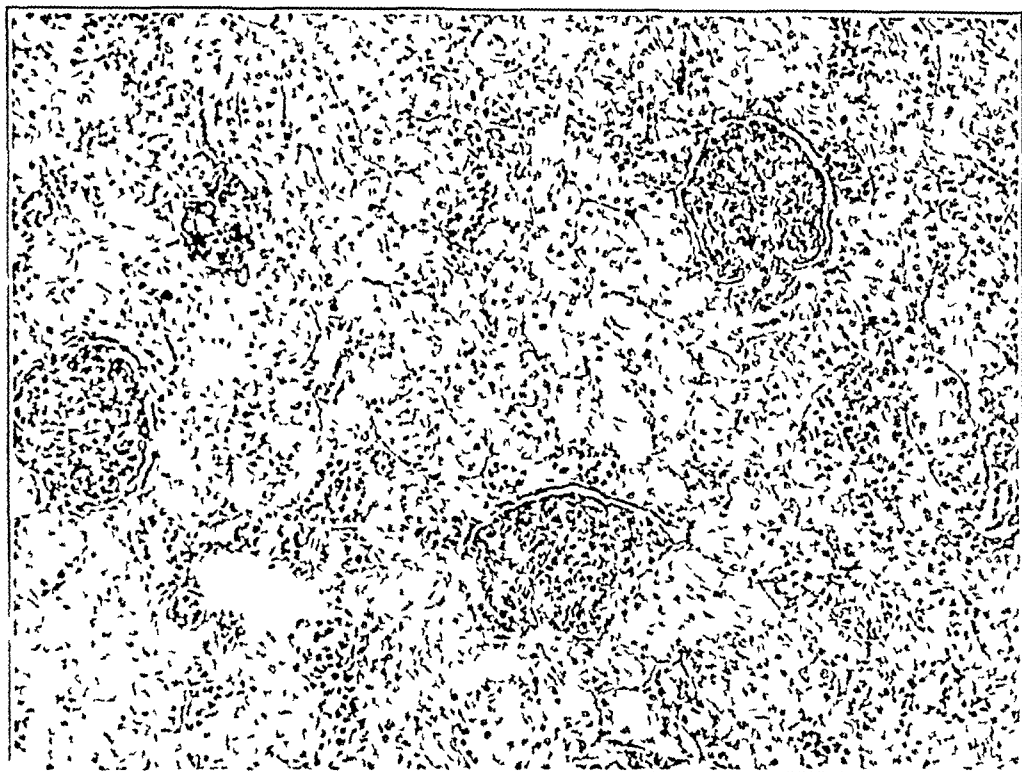


Fig 3—Cortex of right kidney

was negative. Section of one of the larger necrotic and hemorrhagic areas in the cortex showed it to be a typical infarct with complete anemic necrosis in the center and hemorrhage at the border. The tributary artery was found thrombosed. The vascular lesion appeared limited to the arcuate arteries, it was rarely found extending into an interlobular artery or involving the larger vessels entering the hilum of the kidney. The glomeruli throughout the cortex, except in the infarcted areas, were practically normal in appearance (Fig 3). There was no infiltration, swelling nor rupture of the loops. Most of them appeared rather bloodless, while a few were congested. The convoluted tubules were swollen and granular with some vacuolation, indicating marked granular degeneration. The nuclei stained clearly. The lumina of the tubules were almost absent, making identification under low power difficult. The interstitial tissue showed no infiltration and no congestion. Occasionally an area of granular material was found, which was interpreted as edema. A diagnosis of acute and subacute obliterating arteritis limited to the arcuate arteries was made. It was interpreted as being infectious in origin, blood borne and in all probability bilateral.

The patient made a stormy postoperative partial recovery, the temperature gradually dropping to 99-100, the pulse to 100-110. Blood chemistry, October 9, showed a nonprotein nitrogen of 43 mg and creatinin of 2 mg. The patient was allowed to be up and was discharged, October 19, feeling fairly well.

The patient reentered the hospital November 6, on a stretcher, very nervous, apparently in severe pain and quite tender in the right lumbar region. She said that her kidneys had been very active during the preceding week, with nocturia six times, unassociated with pain. She had been feeling fairly well till three days prior to reentrance, when she first noticed shortness of breath and some pain in the back and sides. These symptoms increased rapidly and forced her to bed. Her temperature on entrance was 102, pulse 130, respirations 30, the leukocyte count was 40,000, polymorphonuclears were 82 per cent, red blood cells 4,200,000, hemoglobin 60 per cent. The urine had a specific gravity of 1.018, albumin 2 plus, numerous white blood cells, a few red blood cells and many granular casts. The blood nonprotein nitrogen was 43 mg, creatinin 1.5 mg. The systolic blood pressure was 125, diastolic 90. The pulse was rapid and irregular, temperature around 101 and respirations 25 for the next few days. The output of urine was markedly diminished. With increased elimination, the acute symptoms subsided except that the patient was very weak and still complained of pain and tenderness in the right side of the abdomen and the right lumbar region. The urinary findings cleared considerably, the specific gravity dropping to 1.011, the albumin to 1 plus, there were a few white blood cells and granular casts. A Mosenthal specific gravity test day, November 17, showed fixation at 1.008 with retention, as follows:

	11 a m	1 p m	3 p m	5 p m	7 p m	7 a m
Specific gravity	1.008	1.008	1.008	1.008	1.008	1.007
Amount	75 c c	90 c c	105 c c	90 c c	100 c c	550 c c

The total twenty-four hour intake was 1,650 c c, output, 910 c c. There was slight puffiness of the eyelids, but no general edema. The phenolsulphonephthalein output was less than 5 per cent in two hours. A blood culture was negative. The Mosenthal test day was repeated November 20 with practically the same result:

	11 a m	1 p m	3 p m	5 p m	7 p m	7 a m
Specific gravity	1.008	1.008	1.008	1.008	1.008	1.008
Amount	90 c c	90 c c	110 c c	90 c c	100 c c	540 c c

The total twenty-four hour intake was 1,600 c c, output, 920 c c. The patient left the hospital November 23, against advice, as the prognosis was considered very poor.

The patient was returned to the hospital November 25, on a stretcher, with marked dyspnea and orthopnea, some edema of the feet and slight puffiness of the eyelids. The temperature was 99, pulse 108, respirations 50. There was no heart murmur. The pulse was very weak, the systolic pressure was 100, diastolic 70. Mucous rales were heard in the bronchi. The leukocyte count was 29,600, polymorphonuclears were 84 per cent, red blood cells totaled 3,500,000, and hemoglobin 60 per cent. The urine had a specific gravity of 1.005, albumin 2 plus (14 mg per hundred cubic centimeters) and microscopically showed pus cells 1 plus, a few red blood cells and fairly numerous granular casts. The phenolsulphonephthalein output was less than 5 per cent in two hours. The patient appeared very nervous, although she dozed at short intervals. She vomited considerable mucus and a little blood. There was slight improvement under digitalis therapy, although the pulse remained irregular and rapid—120, and dyspnea and orthopnea persisted. The temperature was subnormal. She complained of pain in her chest, November 27, and a pericardial friction rub was heard. November 28, a systolic murmur was heard over the mitral area. On this day her fluid intake was 2,460 c c and the urine output 990 c c. She became rather drowsy, the lungs filled up with fluid and death occurred November 30 at 3:50 p m. Necropsy was performed at 8 p m.

Necropsy—The body was that of a white, fairly well developed but emaciated woman. The skin was pale, except for many small bluish pigmented areas over both

pectoral upper arm and anterior thigh regions. There was slight edema of the ankles and surgical scars in the right rectus and the right lumbar regions.

The wall of the abdomen was thin, the peritoneum was smooth and glistening, there was no free fluid. There were adhesions to the under side of the right rectus scar, also a separate firm fibrous adhesion laterally between the gallbladder and the anterior abdominal wall. The liver was 8 cm below the costal margin in the midclavicular line, mottled yellow and dark red. Microscopic section of the liver showed a marked congestion about the central veins and corresponding granular degeneration of the liver cords. The portal areas were nearly normal. There was no arterial pathologic change in the sections examined. The gallbladder was of normal size and adhered by fibrous bands to the adjacent pylorus, colon and omentum. The fundus was mottled dark red with punctate hemorrhages and a general intense vascular injection. The wall was thickened and somewhat resistant. The aspirated bile was thin, pale and contained yellow flakes. Cultures of this bile on blood agar were negative.

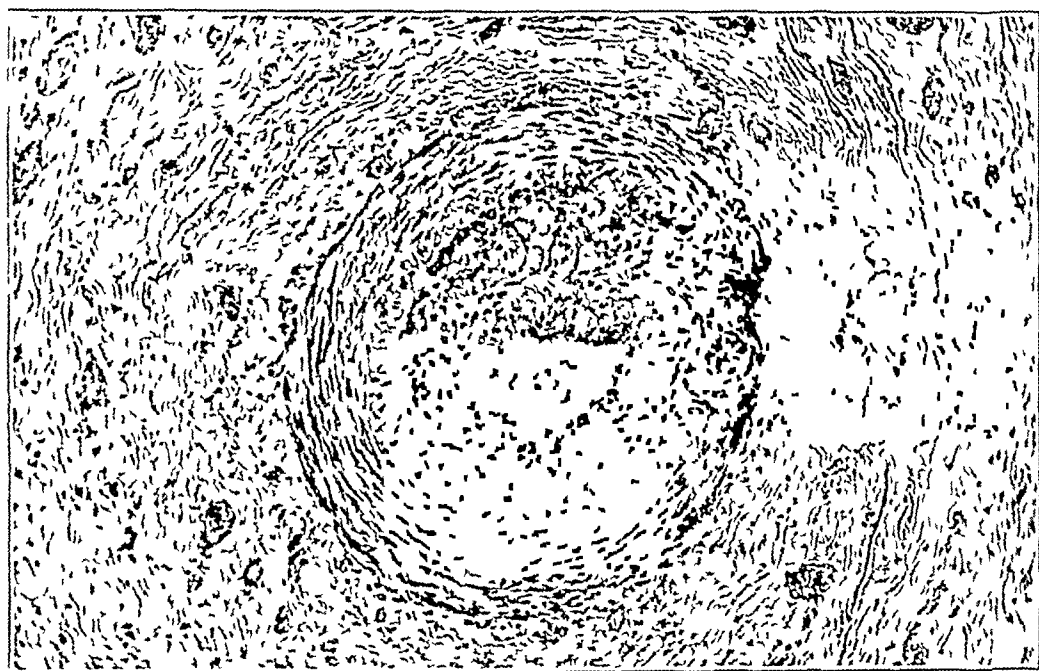


Fig. 4—Cystic artery of gallbladder, showing greatly thickened intima

When the gallbladder was opened, the wall measured 3 mm in thickness, the mucosa was pale and elevated into soft, rounded, bulbous-like folds and projections. This appeared due to a loose swelling of the submucosa. Smears from the fixed submucosa showed an occasional gram-positive diplococcus but no pus cells. Dissection at the neck of the gallbladder showed an even greater thickening and congestion of the wall. Dissection of the hepatic artery and its branches disclosed the cystic branch of darker color, thickened walls and narrowed lumen. This pathologic condition began abruptly at the origin of the cystic artery and did not involve any portion of the right or left hepatic arteries, which were dissected a considerable distance into the liver tissue.

Microscopic sections of the neck of the gallbladder showed the cystic artery with an extreme thickening of the intima about twice the width of the remaining wall (Fig. 4). This thickening was due to a proliferation of fibroblasts and the deposit of an abundant loose fibrillary matrix. The nuclei were mostly round or oval and lenticular, taking a basic stain. The fibrillae also took a basic stain to a considerable degree, in comparison with the collagen fibrils of mature connective tissue. A rare polymorphonuclear leukocyte was found. The lumen

of the artery was a small channel lined by rounded endothelial cells and containing red blood cells. The internal elastic lamina was intact and normal appearing except at one side, where both it and the muscularis were not distinguishable. In this region there was a greater density of fibroblastic cellular tissue with a few inflammatory cells. The muscularis elsewhere was entirely normal. The adventitia likewise was negative for inflammatory cell infiltration but fused imperceptibly with a rather dense white fibrous tissue of the gallbladder wall. In this wall no smooth muscle fibers could be distinguished. There were numerous dilated thin walled blood vessels, evidently veins and capillaries. The mucosa of this region showed a few normal glands but little surface epithelium. The submucosa was moderately infiltrated with mononuclear



Fig 5—Cut surface of left kidney removed at necropsy, showing white infarcts, thickened arteries and narrowed cortex (actual size)

cells. There was almost no infiltration in the gallbladder wall proper. Microscopic sections through the fundus of the gallbladder showed practically the same pathologic condition.

The stomach and small and large intestines showed no gross pathologic change. The pancreas was pale and lobulated. Microscopically, there were very definite arterial lesions in the pancreas, consisting of a marked thickening of the intima by fibroblastic proliferation, variable destruction of the media by fibrosis and slight inflammatory cell infiltration and a marked increase of fibrous tissue in the adventitia. The acinous tissue showed considerable irregular granular degeneration, possibly postmortem. The islands varied likewise from normal to marked degeneration. The spleen was slightly enlarged, dark red, firm and contained a small hemorrhagic infarct. Microscopic sections showed one artery with marked intimal thickening and fibrosis of the wall similar to

that just described in the gallbladder and pancreas. Another artery in the same section showed no pathologic condition. The arterioles of the splenic corpuscles adjacent to the thickened artery showed marked hyaline degeneration and swelling of the entire wall. Others were normal. The infarcted area was of characteristic degenerated appearance. One bordering splenic arteriole had a marked fibrous thickening of the entire wall. A large artery in another section taken beyond the apex of the infarct showed an obliterating intimal fibrous thickening with an organized thrombus in one branch.

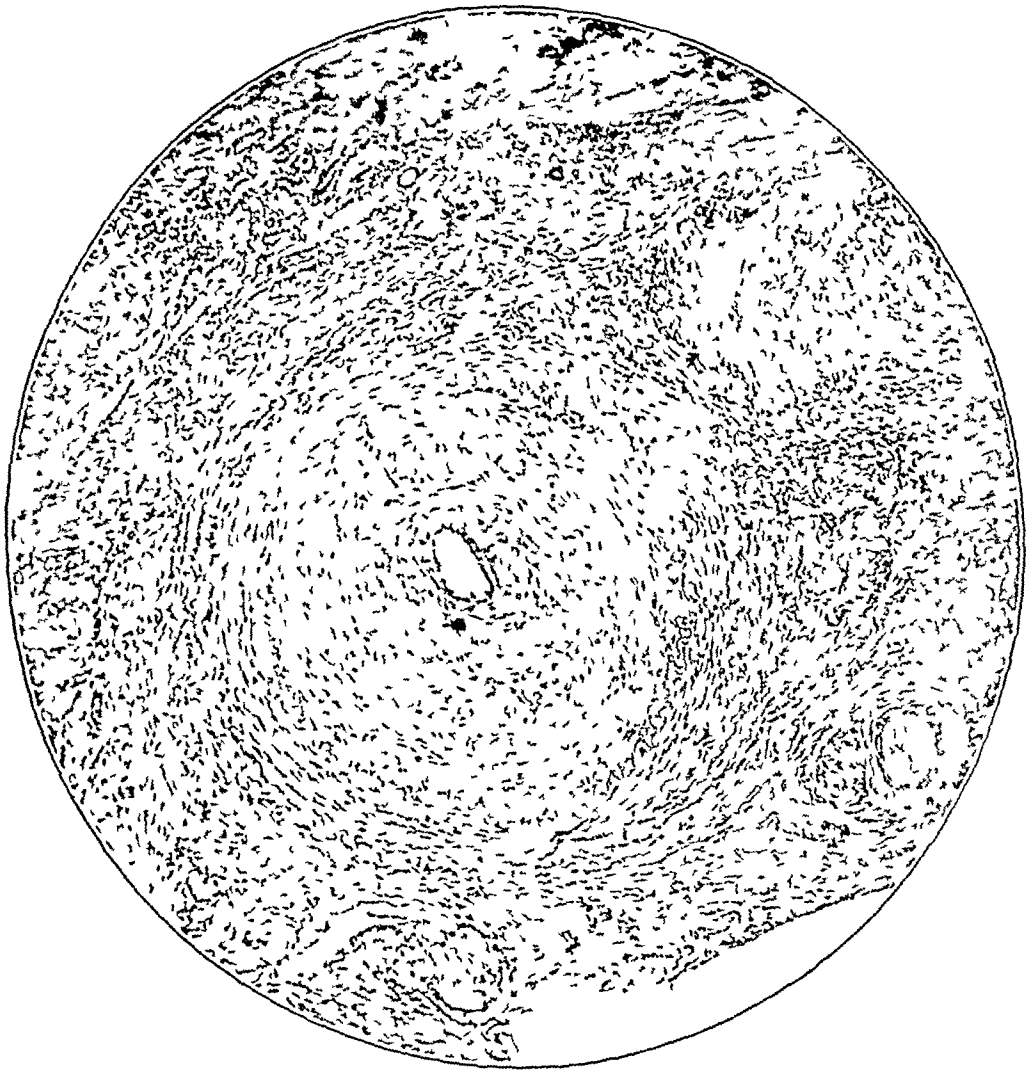


Fig 6—Renal interlobar artery, showing extreme fibrous thickening of intima

The right kidney was absent, its bed filled with fibrous tissue with no evidence of infection. The left kidney was slightly decreased in size, weighing 225 gm, was somewhat adherent in its bed and was mottled with dark red and lighter areas. The capsule stripped easily, leaving an irregular mottled surface. Several yellowish white elevated areas proved by section to be anemic infarcts varying in size from a few millimeters to a centimeter in diameter. The intervening cortex was generally depressed and reddish. The cut surface (Fig 5) showed a distinct thinning of the cortex, the average width being 4 mm, contrasted with a width of 6 mm in the right kidney, removed at operation two months earlier. Also, the line of differentiation between cortex and medulla was somewhat indistinct compared to the right kidney. The larger arteries were dissected in the hilum of the kidney. There was no

pathologic condition of the larger subdivisions, but the lumen could not be followed into the interlobar branches. The walls there appeared thickened, firm and white.

Cross section of one of these arteries showed a pin point red lumen and thick fibrous walls. Microscopically, this artery possessed an enormously thickened fibroblastic intima which measured fully six times the thickness of the muscularis (Fig 6). The very small lumen was lined with a single layer of normal endothelium. There was no inflammatory cell infiltration in the thickened intima. The internal elastic lamina was very distinct. The muscularis was of normal appearance. The adventitia was slightly increased and infiltrated.

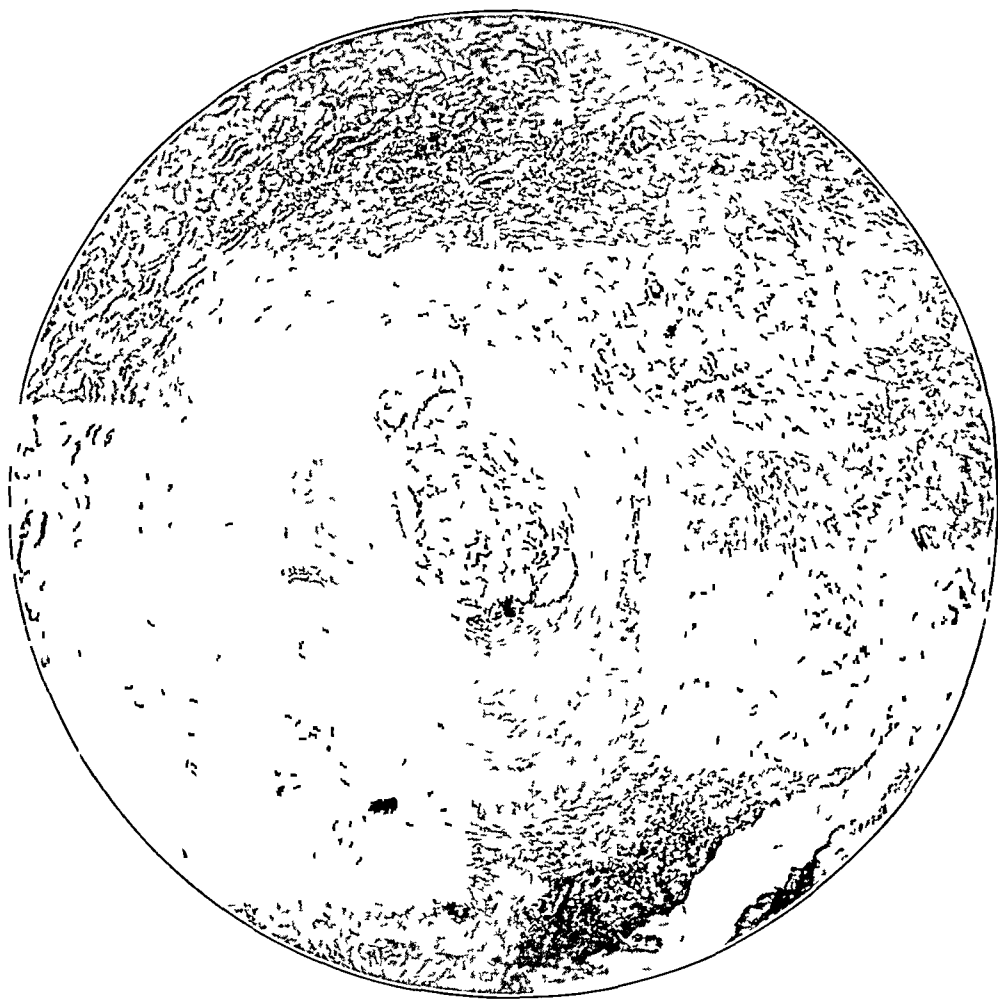


Fig 7—Renal arcuate artery, showing almost complete fibrosis of wall, extremely thickened intima, an organized and canalized thrombus, and moderate perivascular mononuclear cell infiltration.

Microscopic sections of the kidney tissue proper showed the same process of fibroblastic intimal thickening in the arcuate arteries, extending occasionally into the interlobular arteries. In the arcuate arteries there was a greater involvement of the muscularis, which was not distinguishable in places owing to fibrosis and inflammatory cell infiltration. The adventitia also was more involved, thicker and considerably infiltrated. The lumen of some arteries contained organized and canalized thrombi (Fig 7). The extension into interlobular arteries was chiefly as a fibroblastic intimal thickening, although the adventitia

showed some corresponding thickening. This process was irregular, in places involving only one side of a vessel, the other being normal (Fig 8). At no place did it extend into the vas afferens or glomerular loops.

Connective tissue stain (picro-fuchsin) of these pathologic arteries showed the thickened intima taking the characteristic fuchsin of collagen fibrils, although not as intensely as mature white connective tissue. The glomeruli in most regions appeared normal except for congestion. The tubules showed a marked atrophy (Fig 9). The cells of the convoluted tubules were flattened and bounded dilated lumina. The interstitial spaces were widened so that there appeared to be an increase of tissue. Connective tissue stain showed a moderate increase of loose collagen fibrillae. There was very little inflammatory cell infiltration. Irregular areas throughout the kidney showed the effects of more complete vascular obliteration. Where the process was gradual, the glomeruli showed hyaline degeneration or fibrosis and the surrounding tubules showed complete atrophy and disappearance (Fig 19). Their place was taken by a loose connective tissue moderately



Fig 8—Renal interlobular artery, showing unilateral fibrous intimal thickening and corresponding increase of adventitia

infiltrated with mononuclear cells. When the vascular obliteration was more sudden from thrombosis, characteristic complete necrosis of anemic infarcts appeared with beginning organization at the periphery.

Both pleural cavities contained a considerable amount of slightly cloudy straw colored fluid. The serous surfaces were smooth except for adhesions to the mediastinum. The lungs were soft and crepitant throughout. The cut surface exuded frothy fluid. Microscopically, the alveolar septums were slightly thickened and congested. There was no infiltration. The bronchioles were negative. The pericardial cavity contained about an ounce (30 c.c.) of slightly cloudy fluid. The serous surfaces were covered with a thin, white fibrinous layer. The heart appeared slightly enlarged, the wall of the left ventricle measured from 1.5 to 2 cm. in thickness and was firmly contracted. The valves were entirely negative for vegetations or sclerosis. The coronary arteries were dissected and showed no pathologic change. Microscopic sections of the myocardium showed large well preserved cells. There was no thickening of the arteries or perivascular infiltration.

COMMENT

Acute localized inflammation of the smaller arteries of various organs of the body has been described in numerous reports under the name of "periarteritis nodosa." The first clear description of the microscopic pathology of this condition and the application of the name are credited to Kussmaul and Maier¹ in 1866, although the first gross description was given by Rokitsansky² in 1852. Recent excellent reviews of the literature of the subject are given by Lamb,³ 1914, and

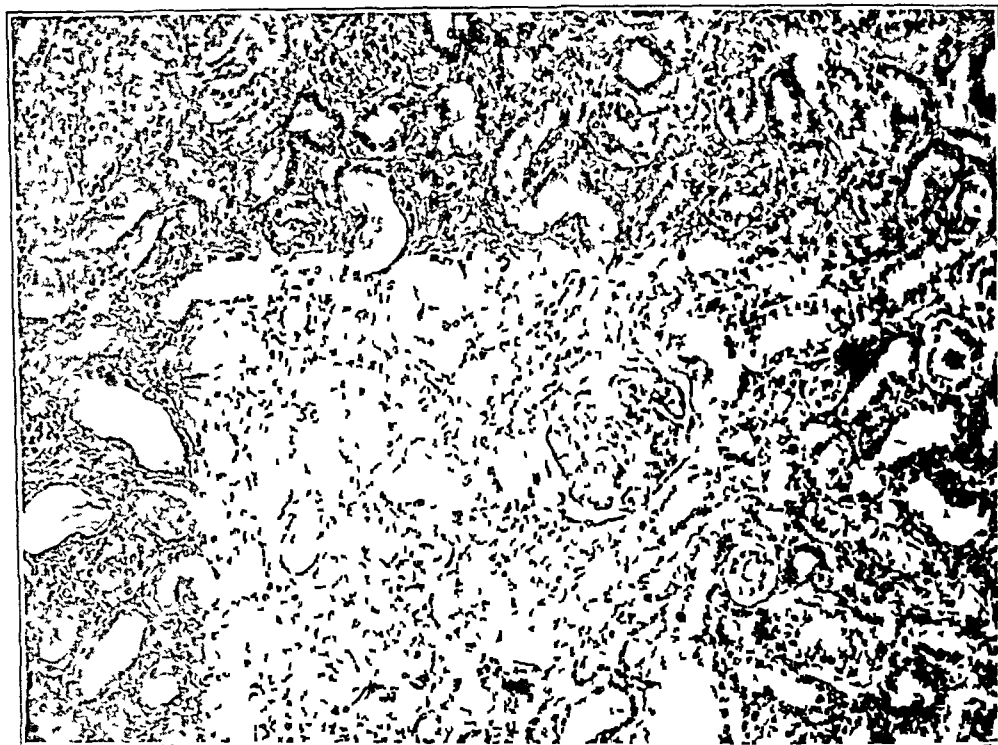


Fig 9—Cortex of left kidney, showing marked tubular atrophy and increase of interstitial tissue

Ophuls,⁴ 1923. Over seventy cases have been recorded chiefly in the European literature. The disease probably is as common in this country but goes unrecognized on account of the difficulty of clinical diagnosis.

1 Kussmaul and Maier. Eine bisher nicht beschriebene eigenthümliche Arterienkrankung die mit Morbus Brighti und rapid fortschreitender allgemeiner Muskellähmung einhergeht, *Deutsch Arch f klin Med* **1** 484, 1866.

2 Rokitsansky. Aneurysmenbildung sammtlicher Arterien mit Ausnahme die Aorta, und der meisten ansehnlichen primitiven Aste derselben, ferner mit Ausnahme der Gehirnarterien, *Denkschr d k Akad d Wissensch, Vienna*, **4** 49, 1852.

3 Lamb, A. R. Periarteritis Nodosa. A Clinical and Pathological Review of the Disease, *Arch Int Med* **14** 481 (Oct) 1914.

4 Ophuls, W. Periarteritis Acuta Nodosa, *Arch Int Med* **32** 870 (Dec) 1923.

and unfamiliarity with its pathology. It has been said to be almost 100 per cent fatal because in all recorded cases the patients have died, but the idea has been expressed by many that both its record of rarity and its high mortality are probably due to the fact that antemortem diagnosis is not made and consequently only fatal cases are recorded.

The clinical picture is that of an acute or gradual onset of obscure sepsis with emaciation, weakness, prostration, fast pulse, marked leukocytosis and frequently intermittent pain in the abdomen. The pathologic findings are well marked nodules in the wall of the smaller arteries with frequent small aneurysms or thrombotic obstruction and infar-

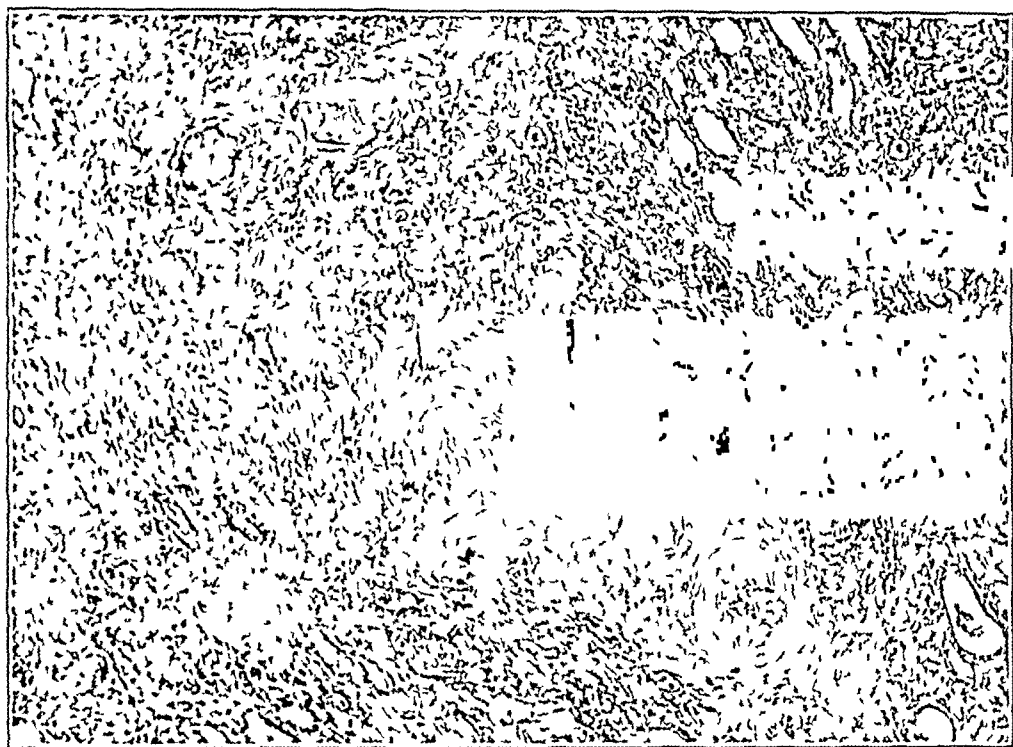


Fig. 10—Cortex of left kidney, showing extensive hyaline degeneration and fibrosis from gradual arterial obliteration.

tion. The coronary arteries of the heart and the smaller arteries of the kidneys are oftenest involved, although the brain and other organs or tissues may be similarly involved. Usually the organs affected are multiple, although the process was found in only one organ in a number of reports. Fishberg⁵ reports a case of six days' duration in which lesions were limited to the kidneys. Other writers call attention to the extension of the arterial inflammation of the kidneys directly into the interstitial tissue causing an interstitial nephritis,¹ or extension into the glomeruli causing an acute or subacute glomerulonephritis.³ Changes in the composition of the urine are usually present and in some cases

⁵ Fishberg, A. M. Zur Kenntniss der Periarteritis nodosa, insbesondere der Histiopathogenese, *Virchows Arch. f. path. Anat.* **240**: 483, 1923.

death has occurred from uremia. The microscopic pathologic condition is an inflammatory cell infiltration usually involving all elements of the artery but characteristically most marked and occasionally alone in the adventitia or perivascular tissue. There has been much discussion as to the site of the primary pathologic condition in the artery wall. The etiology is unknown but a considerable amount of evidence suggests a relationship to streptococcus infection, particularly of the rheumatic group. The rather consistently negative blood, urine and tissue cultures, and the inability to find micro-organisms in tissue sections, as in this case, has prevented general acceptance of streptococcus etiology.

The pathologic condition found in the right kidney in this case, coupled with the preceding obscure septic history of a few weeks' duration, leaves little room for doubt that this was primarily a case of periarteritis acuta nodosa similar to many others reported. The most significant observations, however, were those which developed subsequent to the right nephrectomy. Nocturia and frequency appeared within a month or about two months from the onset of symptoms, and were quickly followed by symptoms of cardiac decompensation. The low kidney phenolsulphonephthalein output, low fixation of specific gravity, slight increase in blood nonprotein nitrogen, albumin, casts and cells in the urine all gave further evidence of a failing kidney function from vascular insufficiency. Death occurred in characteristic manner for primary vascular nephritis, by cardiac decompensation, retention of fluids, late uremic symptoms, subnormal temperature, fibrinous pericarditis and pulmonary edema.

The pathologic condition of the left kidney removed at necropsy, more than three months from the onset of illness and two months from the right nephrectomy, showed a characteristic early stage of chronic primary vascular nephritis. The acute lesions of the arteries of the right kidney were found transformed in the left kidney into early arteriosclerosis. The most prominent feature of these arteries was the great thickening of the intima by young white fibrous tissue. This markedly constricted the lumen and led to anemia of the kidney cortex with atrophy, fibrosis and infarction. The cortex was narrowed, the tubules markedly atrophied and a replacement interstitial fibrosis was under way in varying degree. The symptoms of chronic vascular nephritis presented by this case might be interpreted in sequence from the pathology as follows. Acute vascular infection and infarction in the kidneys led to little urinary change, subsequent gradual fibrotic intimal obliteration of the arteries led to tubular atrophy, with probable loss of reabsorptive power and consequent nocturia and low fixed specific gravity. The rapidity and severity of the arterial obliteration led to cardiac decompensation with less blood flow through the arteries.

and glomeruli and resultant decreased urine output, nitrogen retention and terminal uremia

The kidney pathology of this case suggests that a considerable number of cases of chronic vascular nephritis may have their origin in a mild attack of renal periarteritis nodosa. The kidneys are cited by Lamb³ as being most frequently involved in the reported cases, although Ophuls⁴ placed the coronary arteries of the heart first and the kidney arteries second.

The term "chronic vascular nephritis" is not clearly defined in the minds of many. Ordinarily it has been applied to the senile arteriosclerotic red granular kidney with little functional disturbance. In recent years, added significance has been placed on primary vascular lesions of the kidneys by the work of Jores,⁶ Lohlein,⁷ Gaskell,⁸ Volhard and Fahr.⁹ The first to call attention to the prominence of sclerosed arteries in microscopic sections of chronic nephritis were Johnson¹⁰ and Gull and Sutton.¹¹ Johnson accurately observed the thickened intima of the primary contracted kidney but interpreted it as a greatly hypertrophied internal longitudinal muscular layer. Gull and Sutton¹¹ gave the first interpretation of the primary contracted kidney as due to vascular change. They insisted that the contraction was primarily due to the alterations in the small arteries of the kidney. They held that the main thickening was in the adventitia, that it was diffuse and spread into the surrounding tissues. They named the process arterio-capillary fibrosis.

Jores⁶ revived the views of Gull and Sutton, asserting that the small artery change was a true arteriosclerotic process and part of a general small artery change affecting many organs, particularly the brain. He first described the fat in the wall of these sclerotic arterioles as a characteristic feature. Lohlein⁷ likewise pointed out the enormous thickening of the smallest vessels of the kidney with fat change as the main diagnostic point of primary contracted kidney. Gaskell⁸ studied the same subject with similar conclusions. He subdivided the vascular group of kidney disease into two divisions, the first was the senile

6 Jores, L. Ueber die Arteriosklerose der Kleinen Organarterien und ihre Beziehungen zur Nephritis, *Virchows Arch. t. path. Anat.* **178** 367, 1904.

7 Lohlein, M. Ueber Nephritis nach dem heutigen Stand der pathologisch-anatomischen Forschung, *Ergebn. d. inn. Med. u. Kinderh.* **5** 411, 1910.

8 Gaskell, J. R. On the Changes in Glomeruli and Arteries in Inflammatory and Arteriosclerotic Kidney Disease, *J. Path. & Bacteriol.* **16** 287, 1912.

9 Volhard, F., and Fahr, T. Die Brightsche Nierenkrankheit, Berlin, 1914.

10 Johnson, G. A Case of Chronic Bright's Disease with Rapidly Fatal Sanguineous Apoplexy, *Brit. M. J.* **1** 256, 1872.

11 Gull, W., and Sutton, H. G. On the Pathology of the Morbid State Commonly Called Chronic Bright's Disease with Contracted Kidney (Arterio-capillary Fibrosis), *Brit. M. J.* **1** 620, 1872.

arteriosclerotic kidney due to extension of sclerosis of the aorta and larger vessels into the arterial branches of the kidney, causing disuse atrophy and degeneration of groups of glomeruli and their tubules by obliteration of interlobular arteries. There were no clinical symptoms of kidney disease and the blood pressure was seldom elevated or the heart enlarged. The second division was the "genuine Schrumpfniere," or "primary contracted kidney," which was due to primary arteriosclerosis of the small arteries. This was a part only of a general pathologic complex consisting of hypertrophic changes in the heart and aorta and vascular changes in the kidney and other organs, in particular the brain. It occurred much earlier in life than the senile arteriosclerotic form, usually in patients between 34 and 50 years of age. The conspicuous and important change was the extreme thickening with fat change of the smallest arteries of the kidney, the arteriae afferentes especially, but also of the interlobular arteries.

Volhard and Fahr⁹ made a somewhat similar division of arteriosclerotic kidney changes into (a) the pure arteriosclerotic forms and (b) the combination form, "genuine Schrumpfniere," or primary contracted kidney. In the pure arteriosclerotic group, they included all forms with vascular change that did not enter the glomeruli. They called attention to the well known fact that while sclerosis of the larger and middle sized renal arteries might be a conspicuous feature of the renal histology and might cause puckering and scarring of the renal surface, such sclerosis was not correlated with any typical clinical picture. On the other hand, whenever there was present any considerable thickening of the intima of the smallest renal vessels, there was clinically extremely high blood pressure and marked cardiac hypertrophy.

In these cases the great majority of the glomeruli were normal or almost so and presumably capable of excellent function, and the tubules showed little pathologic change. These cases, in spite of the very high blood pressure, exhibited normal renal function so long as cardiac compensation remained good. This was truly a vascular disease. Death occurred usually from cardiac decompensation or cerebral hemorrhage. The "combination form" of vascular nephritis was that in which in addition to the arteriosclerosis of the type just described, there developed, while cardiac compensation was still good, signs of renal insufficiency often terminating in uremia. Histologically this form showed extensive glomerular atrophy, hyalinization or fibrosis secondary to the primary arteriosclerotic change.

It is thus seen that there has been a growing tendency to interpret the pathologic change of a considerable number of contracted kidneys, so-called chronic interstitial nephritis, as primarily vascular. The origin of the chronic vascular changes so characteristic of these forms of kidney

disease has been stated as unknown by most writers, except for vague references to metabolic toxemias and Volhard's¹² suggestion of a spastic contraction of the small kidney arteries comparable to bronchiole smooth muscle spasm in asthma. A great amount of experimental work has been done attempting to reproduce in animals the characteristic pathologic change of primary contracted kidney of man¹³. Kidney damage has been produced in a variety of ways, leading chiefly to glomerular and tubular damage with secondary chronic inflammatory and fibrotic changes, but the characteristic vascular pathologic change has generally been lacking. The conception of the origin of these vascular changes in specific acute infectious arteritis or periarteritis nodosa as presented by the case reported in this paper, may explain the difficulty of experimental reproduction. The streptococcus or other organism responsible for renal periarteritis nodosa is not easily grown in culture and probably is highly adapted to the human environment.

SUMMARY

1 Renal periarteritis nodosa is considered as an acute vascular nephritis leading in nonfatal cases to chronic renal arteriosclerosis and chronic vascular nephritis.

2 The term "chronic vascular nephritis" is applied to a large group of contracted kidneys in which the primary pathologic condition is in the arteries and the secondary pathologic change is due to gradual arterial obliteration.

12 Volhard, F. Die Doppelseitigen haemotogenen Nierenkrankungen, Berlin, 1918.

13 Leiter, I. Experimental Chronic Glomerulonephritis, Arch. Int. Med. **33** 611 (May) 1924.

WHEN DO LUNGS RETURN TO NORMAL FOLLOWING EXPOSURE TO WAR GASES?¹

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The use of toxic gases during the recent World War introduced a new subject in medicine. The clinical effects and the acute pathologic lesions of these gases have now been quite thoroughly investigated, but very little is known concerning what chronic lesions may remain after all symptoms have subsided. The need for further knowledge on this subject is apparent to all concerned in the care of sick veterans or in the fixing of compensation for soldiers who have been gassed. Because there is little opportunity to gain this knowledge from studies on soldiers who were the victims of gas attacks, this series of experiments on animals has been carried out.

We have attempted to determine whether pathologic lesions persist in the lungs of dogs which have been gassed but which have recovered from all symptoms. We had hoped to study the pathologic physiology as well as the pathologic anatomy of these animals, but have been prevented from doing so by our inability to measure the working capacity of dogs by means of the treadmill or other device.

METHOD

All the dogs used were gassed with the minimal lethal dose of the gas in question. Some dogs survived this dose, but were sick for periods of time varying from a few days to a month. They were considered recovered after their temperature, pulse and respiration had become normal and all symptoms, such as coughing, depression and general lack of well being, had disappeared. These dogs were then kept in kennels having large yards, so that they could exercise and live under approximately normal conditions. They were killed at varying intervals of time after recovery and carefully studied in order to see if any pathologic lesions remained as a result of the gassing.

Various methods of killing animals that had recovered were tried in order to find a method which in itself would cause the least variation from the normal in tissue structure. At first we used injections of potassium cyanid into the heart, but it was found that this caused an extreme grade of congestion in the lungs. Chloroform also caused lung congestion and, besides, theoretical objections might be raised to the

¹From the Pathological Section, Medical Research Division Edgewood Arsenal

use of any inhaled vapor. Shooting or a blow on the head caused confusing hemorrhages and objectionable agonal struggles. We finally adopted the method of injecting a saturated solution of magnesium sulphate into the heart, and have used it in practically all our experiments. This causes very little congestion of the lungs, even when injected into the right side of the heart. To avoid this entirely, we have as a routine procedure injected 10 c.c. of the solution into the left ventricle. This makes it necessary for the solution to travel through the entire systemic circulation before it reaches the lungs, where it produces little or no change. Death is practically instantaneous without agonal convulsions, which, if severe enough, are in themselves often the cause of hemorrhage. We have therefore an ideal method of producing death, as it does not alter the pathologic condition that existed in the living animal.

The lungs of dogs are rather delicate mechanisms and will permit of very little handling without structural alterations. We were therefore troubled in our early work with lesions that we felt must be artefacts. Atelectasis was the main lesion thus encountered, and was almost constantly encountered in spite of the most careful handling, and the use of very sharp knives in cutting the blocks for fixation. In order to avoid this difficulty, we have employed the method of removing both lungs with the trachea and running Zenker's fluid into the trachea until the lungs are normally expanded, and at the same time immersing them in a vessel containing Zenker's fluid. By this method fixation is complete and almost instantaneous, and after the lungs have remained in Zenker's fluid a sufficient time, blocks can be cut for section without danger of producing artefacts. It is best to run the fixative into the trachea until the lungs are pretty well distended before immersing them in the vessel containing the fixative, otherwise the subpleural portions will be fixed in their collapsed state before the intratracheally injected fixative reaches these parts, and under the microscope these regions give the appearance of marginal atelectasis. By carefully carrying out this method, however, it is possible to bring the danger of artefacts to a minimum.

We have studied principally dogs which had recovered from the effects of phosgene, mustard, lewisite, chlorine, chlorpicrin, and methyl-dichlorarsin. There is a total of 313 dogs in the series. The effects of each gas are considered separately below.

The dogs that recovered and later died in the kennels and those which were killed in the kennels by other dogs have been included in the series along with those which were killed at definite stated intervals after the cessation of symptoms. This has been done for the sake of completeness and in order to be sure not to vitiate our results. Most of the

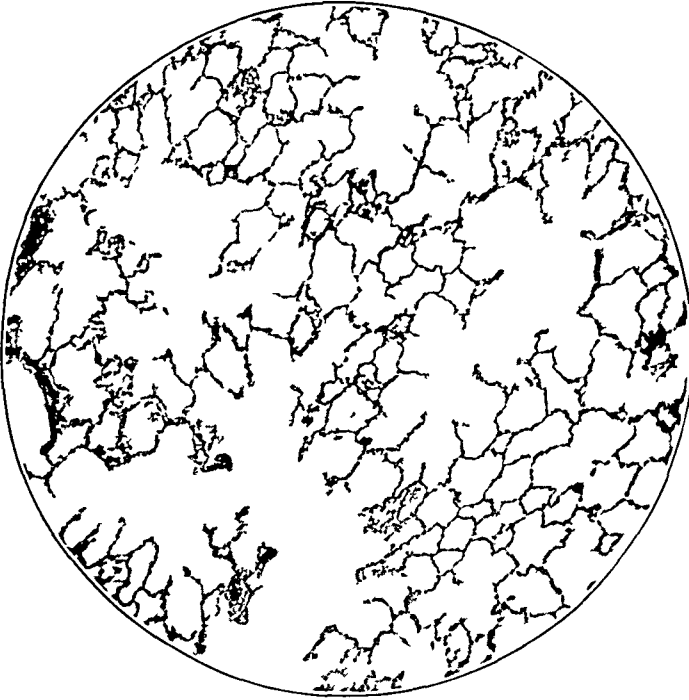


Fig 1—Normal lungs in dog two weeks after recovery from phosgene

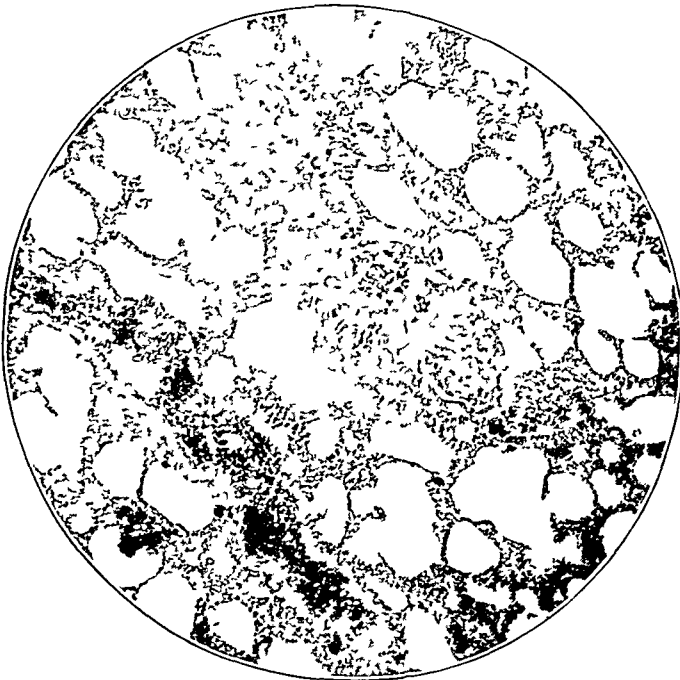


Fig 2—Bronchi plugged with exudate, desquamated epithelial cells and debris in dog that died three weeks after recovery from phosgene

dogs dying in the kennels died of some acute lung condition which may or may not have had any relation to the previous gassing. Of the ordinary run of stock dogs kept in the kennels, about 10 per cent die or are killed in the course of a year. It will be seen that the percentage for our dogs that recovered from gassing is much higher (28.1 per cent). That there is some relationship between previous gassing and death in the kennels is thus clearly established. Most of this high mortality rate, however, occurred within the first two months after recovery from gassing, 39.8 per cent of the total number of recovered dogs in the

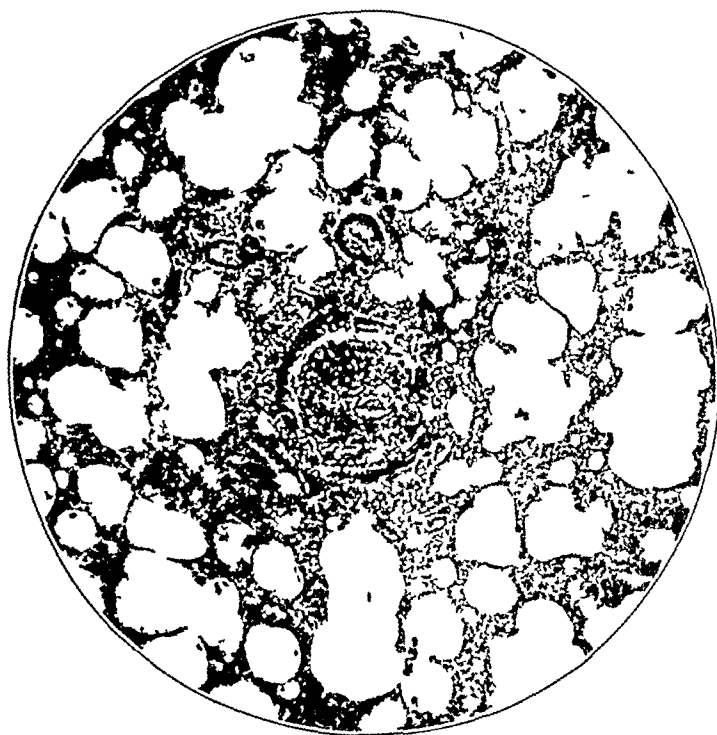


Fig. 3—Obliterative bronchiolitis in dog that was killed by other dogs three weeks after recovery from phosgene

kennels dying within the first month, 33 $\frac{1}{3}$ per cent during the second month, and only 16.4 per cent during the period from the end of the second month to the end of the fifteenth month.

PHOSGENE

Ninety-five dogs that recovered from gassing with phosgene were studied. Sixty-one of these were killed with magnesium sulphate at intervals varying from two weeks to fifteen months after recovery. Thirty-four died or were killed in the kennels at intervals of from one week to eight months after recovery.

Gross Pathology—Sixty-eight of the series appeared normal in the gross. Of the remaining twenty-seven, three showed empyema and

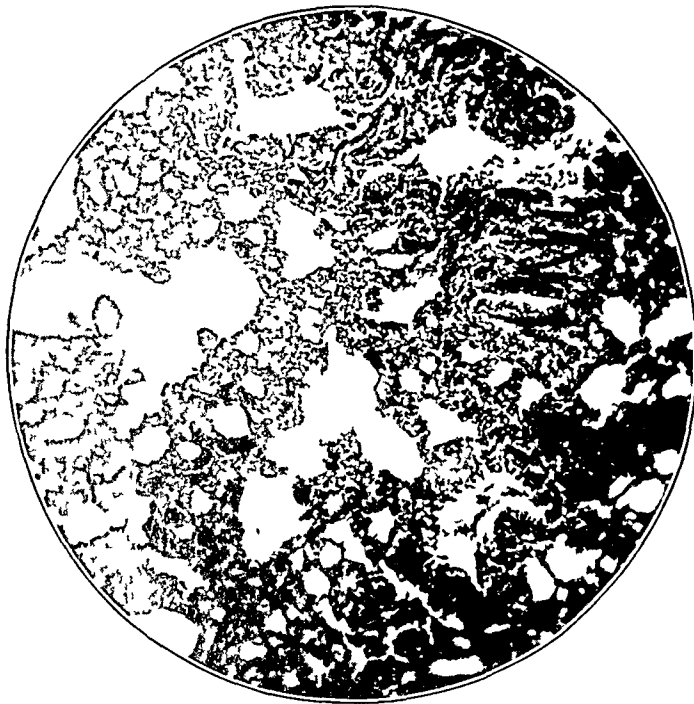


Fig 4—Chronic bronchitis and partial atelectasis due to plugged bronchi in a dog killed five weeks after recovery from phosgene



Fig 5—Patch of organization in dog killed eighteen weeks after recovery from phosgene

two pneumonia. The others had such lesions as congestion, hemorrhage, edema and emphysema in varying degrees of intensity. Most of these gross lesions were found in dogs that died.

Microscopic Findings—In three of ten dogs that were killed or died two weeks after recovery, the lungs were normal microscopically. There was no definite point in the series at which it could be said that all pathologic lesions had disappeared, and the majority of the lungs were normal. However, of the ninety-five dogs studied, in twenty-one the lungs were normal, and in twenty-four more were almost normal, having only small areas in which there were lesions of minor degree. Of the

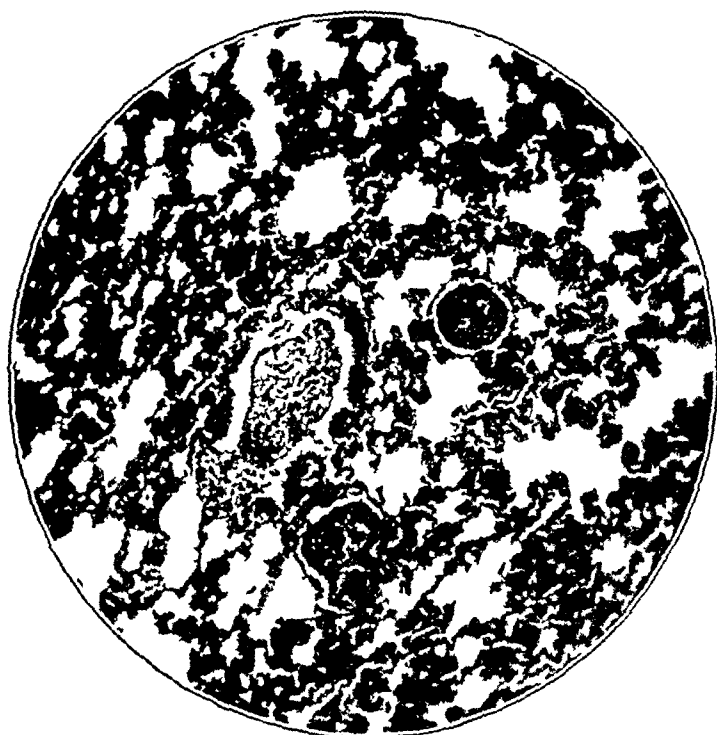


Fig. 6—Chronic obliterative bronchiolitis in dog that died eight months after apparent recovery from phosgene.

lesions encountered in the early weeks after recovery, the predominating one was a plugging of the bronchi with desquamated epithelial cells and debris and atelectasis of the adjacent lung tissue. This gradually gave way to obliterative bronchiolitis and small patches of organization as the principal lesions. Small hemorrhages were often found around the patches of organization. On the whole, however, the appearance of the lungs took on a more and more normal aspect as the time from the date of recovery increased. As would be expected, a much larger area in which there was pathologic change was encountered in dogs dying in the kennels than in those killed with magnesium sulphate (Figs. 1 to 6).

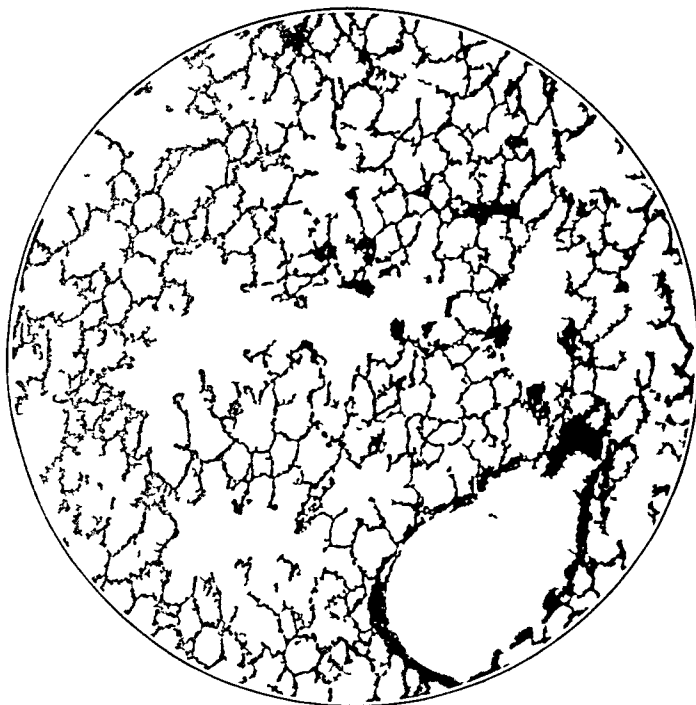


Fig 7—Normal lungs in dog killed at time of recovery after being sick for four weeks from mustard



Fig 8—Hypertrophied bronchial epithelium and dilated mucous glands in wall of bronchus of dog killed six weeks after recovery from mustard

MUSTARD

There were fifty-eight dogs in the series given mustard gas. Of these forty were killed with magnesium sulphate at intervals varying from the actual time of recovery to eight months after recovery. Eighteen died or were killed in the kennels at intervals of from one week to nine months after recovery.

Gross Pathology—Forty-six of the fifty-eight dogs studied appeared normal in the gross. Of the remaining twelve, one had pneumonia. The others showed such lesions as congestion, hemorrhage, edema, atelectasis and emphysema. As in the case of phosgene, nearly all the gross lesions occurred in dogs that died.

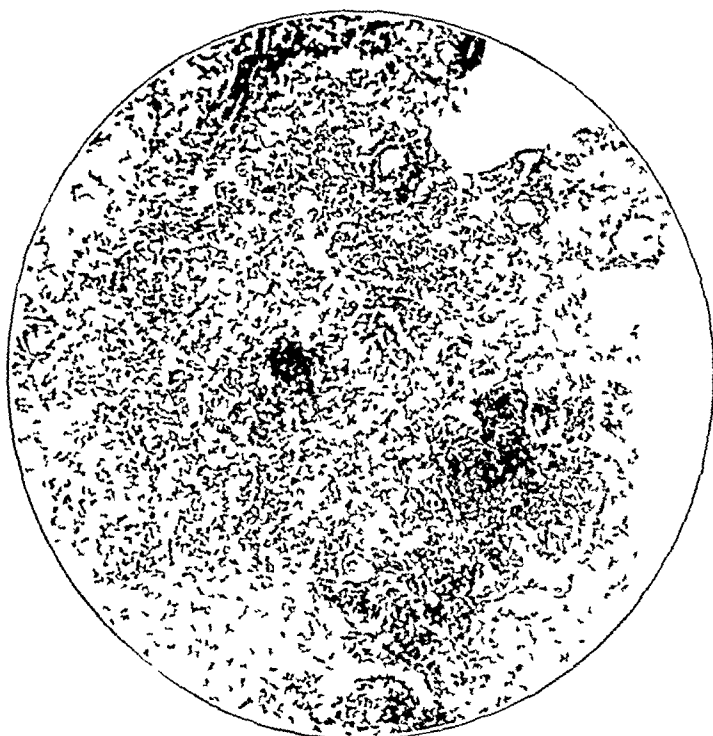


Fig 9—Normal lungs except for few patches of organization surrounded by minute areas of bronchopneumonia in dog that never really recovered from effects of mustard gas. The dog was discharged from the hospital at the end of twenty weeks still having a hoarse cough and bad eyes.

Microscopic Findings—The only dog killed at the actual time of recovery had lungs that were entirely normal. Of eleven dogs killed from two to three weeks after recovery, three had normal lungs. Of six dogs killed four weeks after recovery, four had normal lungs. Of six dogs killed six weeks after recovery, three had normal lungs. From eight weeks on practically the only lesions encountered were small patches of organization. The early lesions, as in the dogs that recovered from phosgene, consisted principally of the plugging of bronchi with debris and atelectasis of the adjacent lung tissue. In most cases these

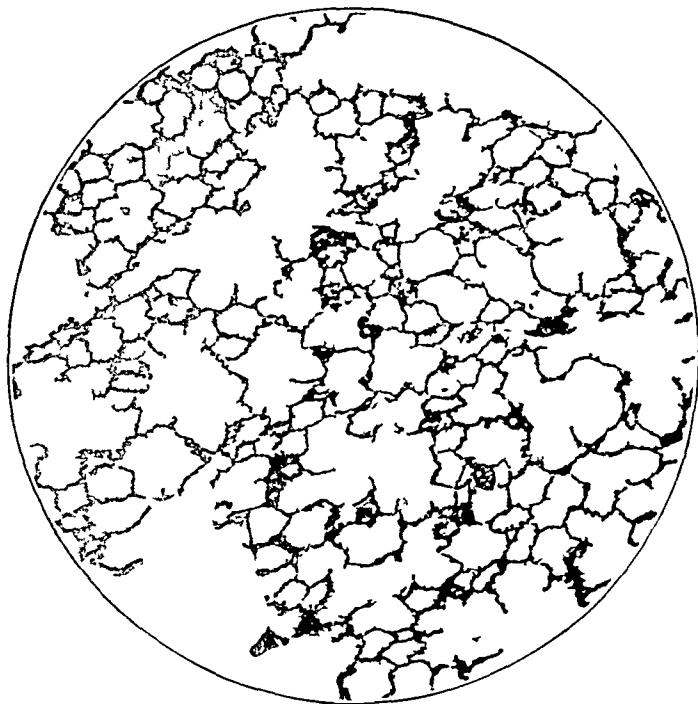


Fig 10 Normal lungs of dog killed five weeks after recovery from lewisite

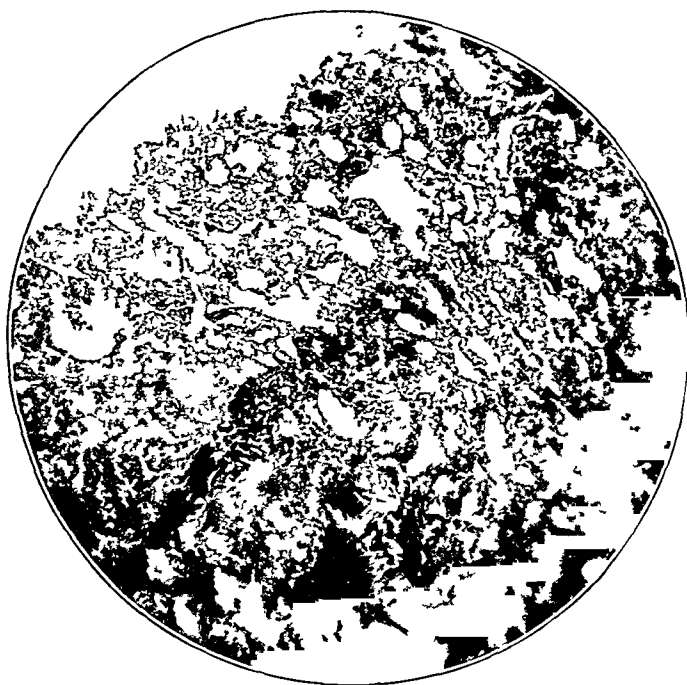


Fig 11—Plugging of bronchus with desquamated epithelial cells and debris in dog that died ten weeks after recovery from lewisite

lesions occupied very limited areas of lung tissue. Occasionally, areas of chronic inflammatory reaction were encountered. Congestion, hemorrhage and edema were often found in the dogs dying in the kennels (Figs 7 to 9).

LEWISITE

There was a total of sixty dogs in the series given lewisite. Forty-five of these were killed with magnesium sulphate at intervals varying from two to twenty-one weeks after recovery. Fifteen died or were killed in the kennels at intervals of from one to ten weeks after recovery.

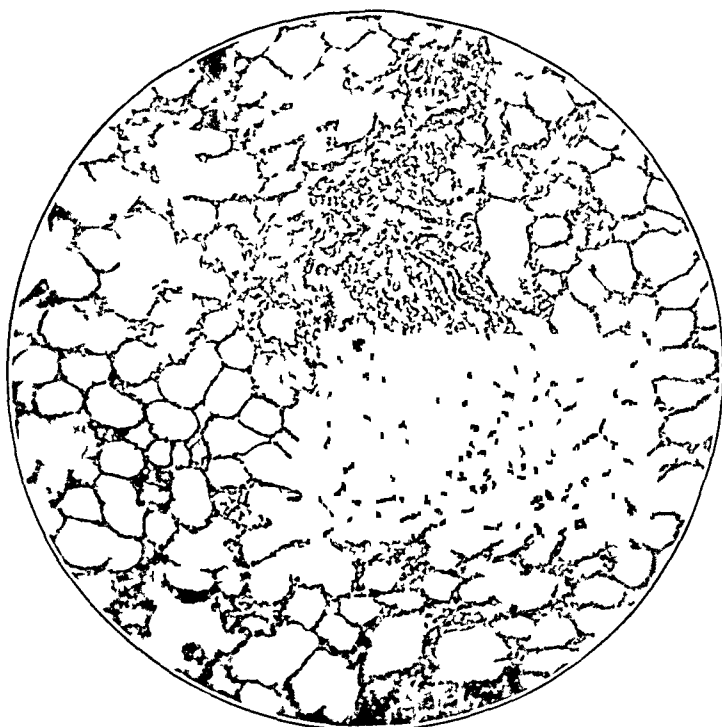


Fig 12—Organizing oblitative bronchiolitis in dog that died ten weeks after recovery from lewisite

Gross Pathology—Forty-five of the sixty dogs studied appeared normal in the gross. These were the dogs killed. Of those dying, fifteen in number, four had pneumonia and one bronchitis. The others showed varying degrees of congestion, hemorrhage, edema, atelectasis and emphysema.

Microscopic Findings—Lesions similar to those encountered in the early weeks after recovery in the phosgene and mustard series were found in this series also during the first five weeks. The sixth week in this series, however, marks a definite point at which the pathologic lesions disappear, except for a few chronic changes similar to those spoken of above, and the more acute changes found in those dogs which die in the kennels (Figs 10 to 12).

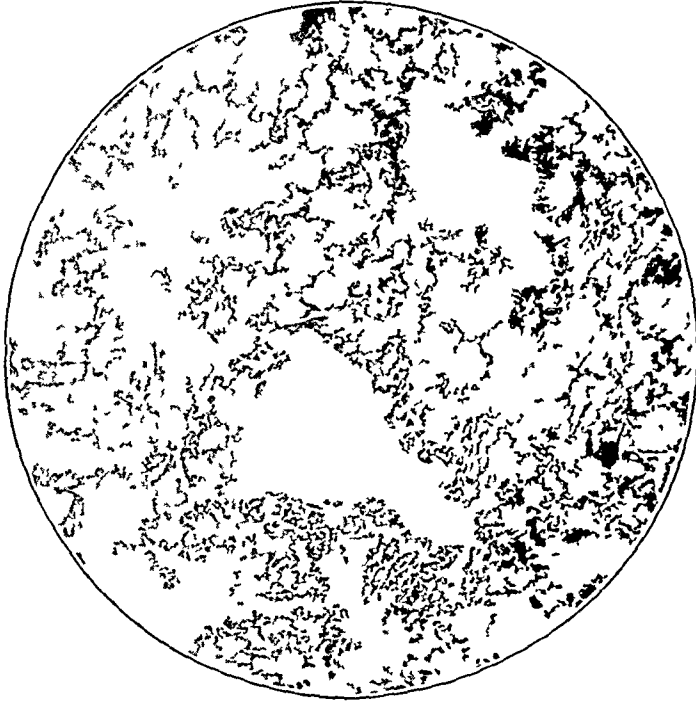


Fig 13—Marginal atelectasis and minute hemorrhages in almost normal lungs in dog killed one week after recovery from chloroform

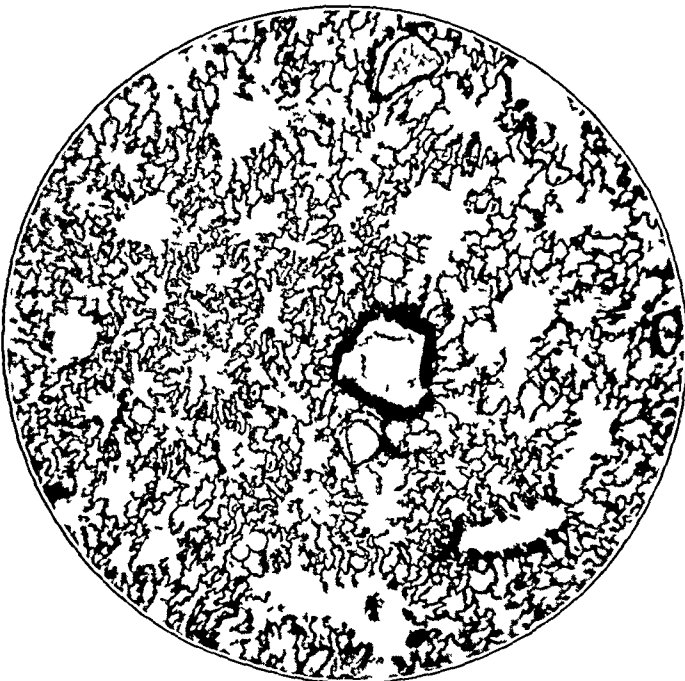


Fig 14—Normal lungs in dog killed four weeks after recovery from chloroform

CHLORIN

There were only eleven dogs in the series given chlorin—too small a number from which to draw any definite conclusions. Seven of the eleven were killed with magnesium sulphate at intervals varying from one to twenty weeks after recovery. Four died or were killed in the kennels at intervals of from two to four weeks after recovery.

Gross Pathology—Nine of the eleven showed no gross lesions. Of the remaining two, one had pneumonia and the other bronchitis. Both of these died in the kennels.

Microscopic Findings—There were no absolutely normal lungs in the six dogs examined at necropsy in the first six weeks after recovery,

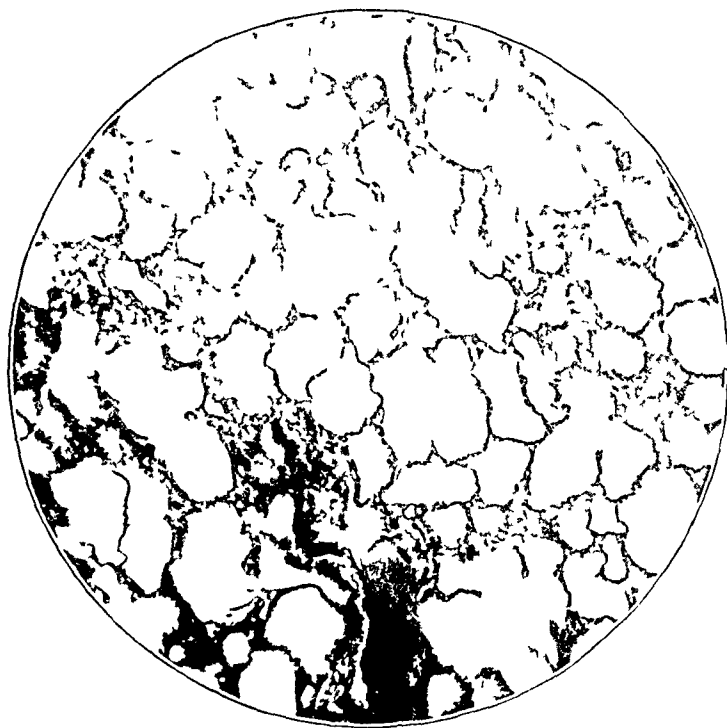


Fig 15—Small areas of organization in dog killed twenty-six weeks after recovery from chlorpicrin

but this number included the four that died in the kennels. There were no necropsies on animals between six and thirteen weeks after recovery. Of the five examined at necropsy from thirteen to twenty weeks after recovery, three were normal. One examined at necropsy at fourteen weeks showed some old scars, and one at fifteen weeks had small areas of organization and hemorrhage (Fig 13).

CHLORPICRIN

There were forty-eight dogs in the series given chlorpicrin. Eight died in the kennels, and forty were killed at intervals ranging from one to twenty-six weeks after apparent recovery.

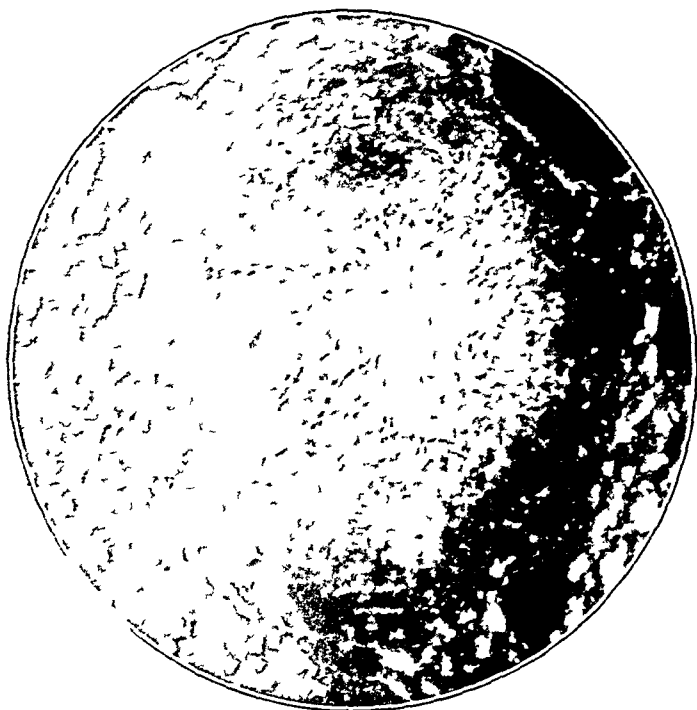


Fig 16—High magnification of chronic proliferative bronchiolitis in same lung as that shown in Figure 15

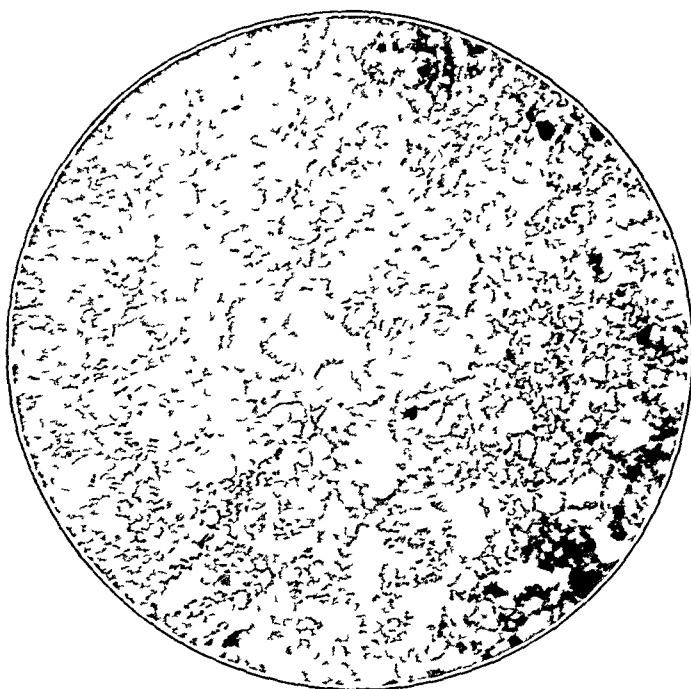


Fig 17—Partial return of lungs to normal in dog killed four weeks after recovery from methyldichlorarsin

Gross Pathology—There were no gross lesions, except for an occasional case of congestion, atelectasis or emphysema occurring in dogs that died

Microscopic Findings—The lungs of dogs killed from five to ten weeks after recovery showed an almost complete return to the normal. The dogs killed prior to this time showed about the same type of lesions as those occurring in the early weeks after recovery from the other gases. Dogs killed later showed occasional patches of organization and chronic bronchitis in some cases (Figs 14 to 16)

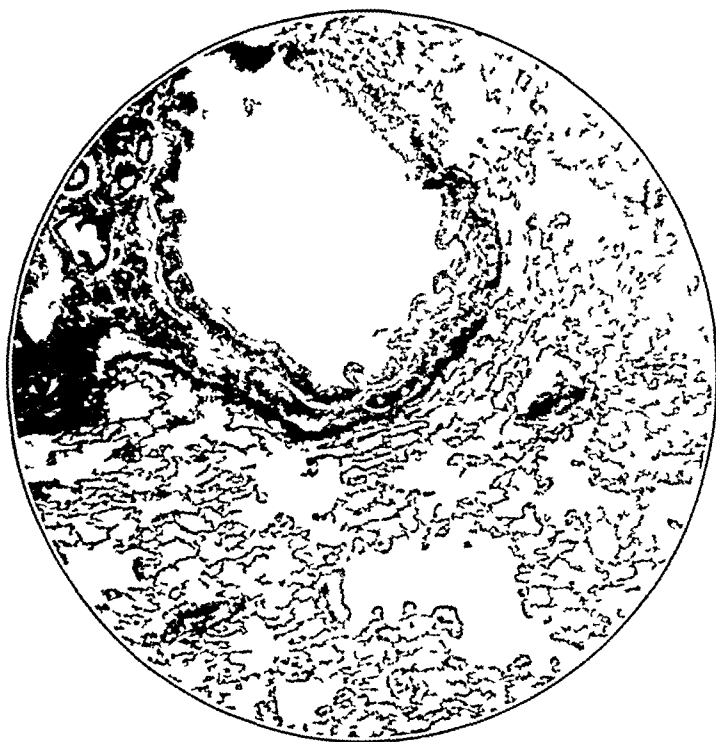


Fig 18—Normal lungs in dog killed eight weeks after recovery from methyl-dichlorarsin

METHYLDICHLORARSIN

Forty-one dogs in the methyldichlorarsin series were examined at necropsy at intervals ranging from two to sixteen weeks after recovery. Nine of these died or were killed in the kennels. The rest were killed in the usual manner.

Gross Pathology—In the thirty-two dogs sacrificed, there were no gross pathologic lesions. Of the remaining nine, one had pleurisy with adhesions, three had pneumonia, one organizing pneumonia, three purulent bronchitis, and one chronic peribronchitis.

Microscopic Findings—Most of the animals had returned to normal within eight weeks after recovery, but some chronic changes were found up to sixteen weeks (Figs 17 and 18)

SUMMARY

A number of dogs were exposed to approximately the lethal concentration of each of the following gases phosgene, mustard, lewisite, chlorine, chloropicrin and methyldichlorarsin. The dogs that recovered from the effects of the gases were discharged from observation on the cessation of symptoms, and were kept in the kennels to be killed at varying intervals of time from the date of recovery, in order that the pathologic lesions still remaining in the lungs might be studied. Of the recovered dogs, almost a third died in the kennels or were killed in the kennels by other dogs. This makes the mortality almost three times as high among the recovered gassed dogs as among the ordinary run of stock dogs in the kennels. It seems conclusive, therefore, that there is some connection between these deaths and the previous gassing of the animals. That no such parallel exists in men who were gassed during the World War is shown equally conclusively by the histories of men who were gassed during that conflict. The reason for the difference is probably that the soldiers had the best hospital care and treatment during the period of illness following the gassing, and equally good care for still further periods in convalescent camps, while the dogs received no treatment whatsoever, and after their discharge from the dog hospital, were exposed to rigors of weather and climate and the savage attacks of stronger dogs in the kennels.

The pathologic lesions found in the lungs of the dogs that died in the kennels were varied in number. In a few cases the lungs were normal, and such dogs probably died of starvation, being too weak or apathetic to compete for their food. In a number of other instances, pneumonia was the cause of death. In the majority of cases, however, such lesions as congestion, hemorrhage, edema, plugging of the bronchi with debris and atelectasis were found.

The dogs that were killed at varying intervals after recovery may be divided into three groups as follows: (1) those in which the lungs were normal, (2) those which had more or less acute pulmonary lesions, and (3) those which showed chronic lesions. Normal lungs were found in a few dogs that were killed at the time of recovery, and the percentage of normal lungs gradually increased from this time to the end of the period of observation (fifteen months from date of recovery). However, the percentage of normal lungs in the first five to eight weeks after recovery was not very large, while after this period normal lungs were in the large majority. The more or less acute lesions that occurred in the first five to eight weeks after recovery consisted principally of edema, desquamation of alveolar epithelium, plugging of bronchi with debris, atelectasis, occasional patches of chronic inflammatory reaction and an occasional pneumonia. The chronic lesions occurred between the

period from five to eight weeks after the date of recovery and the end of the period of observation, and consisted principally of small patches of organization, often associated with minute hemorrhages, chronic bronchitis and peribronchitis and organizing obliterative bronchiolitis.

It should be made clear that the pathologic lesions encountered in the group of dogs that were sacrificed at various intervals of time after recovery were limited in extent and only discovered microscopically. In practically all of these dogs the lungs appeared normal in the gross. This is in sharp contrast with the dogs that died, in nearly all of which gross lesions were discovered at the necropsy table.

CONCLUSIONS

- 1 In the great majority of dogs gassed at lethal concentrations, no pathologic lesions can be demonstrated from two months to a year after recovery.

- 2 In a minority of instances, permanent lung damage is done, but this damage is not widespread, is generally confined to small areas, and is generally in the form of small patches of organization, thickening of the bronchial walls with loss of elasticity, or occasional closing of the bronchioles by organized exudate.

- 3 All such damage will ultimately result in fibrous tissue. This small amount of scar tissue can hardly affect the functional efficiency of the lungs.

- 4 Since the lungs of dogs are more delicate than those of human lungs, it is reasonable to conclude that in the vast majority of surviving human patients, who were not gassed so severely and who receive infinitely better care in hospital, all lesions have disappeared within a short while after gassing, and that the small amount of fibrosis remaining in the minority of cases can hardly be a cause of serious disability.

FORMALDEHYD POISONING

WITH REPORT OF A FATAL CASE *

B S KLINE, M D

CLEVELAND

Although formaldehyd has been extensively used in a number of industries since its discovery in 1867 by Von Hoffmann, there are on record, including the one at Mt Sinai Hospital reported here only twenty-seven cases of formaldehyd poisoning

Of these twelve patients (seven males and five females) died in from twenty or thirty minutes to four weeks In the case of the males, formaldehyd was taken by mistake for whisky or water In the case of the females, it was taken with suicidal intent

The amount of formaldehyd ingested in the fatal cases varied from a few drops to 3 ounces (89 c c) of concentrated solution The largest amount from which a patient recovered (case of Hale) was 120 c c of concentrated commercial formaldehyd

Below is a summary of the clinical and pathologic records of the fatal cases The first four are quoted from MacLachlan,¹ who reviewed the literature to 1909 and in his report summarized the clinical features of formaldehyd poisoning

REPORT OF CASES

CASE 1 (Reported by Bock² in 1899) —A man, aged 26, took 3 ounces (89 c c) of solution of formaldehyd U S P (formalin 100 per cent) and died in thirty-two hours

Clinical Course —The patient immediately vomited blood tinged mucus and had severe epigastric pain Demulcents and apomorphin were given at once resulting in free emesis The patient continued weak and vomited repeatedly Sixteen hours later the pulse first weakened The patient remained in status quo until the twenty-ninth hour, when the heart failed rapidly At the thirty-second hour, there was cyanosis, coma and then death

Pathology —The lower end of the esophagus showed marked erosion The stomach wall and duodenum were very much congested and cut like leather Microscopic examination was not made Other findings were not abnormal

CASE 2 (Reported by Levison³ in 1904) —A man, aged 60, took 3 ounces (89 c c) of solution of formaldehyd U S P (formalin 100 per cent) and died in twenty-nine minutes

Clinical Course —The patient was found writhing in pain and unable to speak He did not vomit even after three-tenths grain (0.018 gm) of apomorphin Lavage was attempted, but the tube could not be passed on account of spasm of the pharynx The patient died of cardiac failure in twenty-nine minutes after taking the poison

* From the Laboratory Service of Mt Sinai Hospital

1 MacLachlan Cleveland M J 8 606, 1909

2 Bock Fort Wayne M J 19 249, 1899

3 Levison, L A J A M A 42 1492 (June 4) 1904

Pathology—The esophagus, stomach and small intestine were literally "hardened"

CASE 3 (Reported by Bose⁴ in 1905) —A man, aged 47, took 3 ounces (89 c c) of solution of formaldehyd U S P (formalin 100 per cent) and died in thirteen hours

Clinical Course —The patient was found shortly after taking the poison, unable to speak, with hands on abdomen and apparently in great pain. He did not vomit before admission to the hospital. After a lavage he was able to talk rationally. He started to vomit almost at once and continued all that night the vomitus being blood tinged. The patient gradually sank but developed no other symptoms and died the next day, thirteen hours after the ingestion of the poison.

Pathology—The esophageal and gastric mucosa was intensely congested, as was that of the small and large intestines. Some slight changes in the parenchymatous organs were noted.

CASE 4 (Reported by Palmer in 1906) —A man, aged 29, took about 7½ ounces (222 c c) of solution of formaldehyd U S P (formalin percentage not given), and died in three days.

Clinical Course —Shortly following ingestion, the patient experienced intense abdominal pain and collapsed. When admitted to the hospital, he was tender over the epigastrium. He vomited blood tinged fluid, was slightly delirious and had a marked feeling of constriction of the throat. He was given a dilute solution of ammonia followed by demulcents. The next day his mind was wandering but he was in no special pain. Two days later, he became markedly delirious, noisy, and at times maniacal. During one of these attacks, his pulse failed, his breathing became shallow, and his heart stopped.

Pathology—The blood everywhere was found dark colored and fluid. The mucous membrane of the lower end of the esophagus and the gastric mucosa were tanned, and the parenchymatous organs were somewhat congested.

CASE 5 (Reported by Ely⁶ in 1910) —A boy, aged 3 years, took a few drops of solution of formaldehyd U S P (formalin 100 per cent) and died in about forty-eight hours.

Clinical Course —The patient was immediately seized with a paroxysm of coughing and choking, but the attack soon ceased. The child's mouth was rinsed out thoroughly with water, and he was soon at play as if nothing had happened.

On the next day, there was a hoarse laryngeal cough with accelerated and labored breathing. The pulse and temperature were normal and in every respect the child seemed to be in perfect health. A laryngeal application of epinephrin was employed with temporary benefit. Inhalations of steam were advised, and frequent teaspoonful doses of olive oil were ordered to allay any pharyngeal or esophageal irritation. Fifteen hours later, the child was deeply cyanosed and showed every sign of laryngeal obstruction. Repeated applications of epinephrin were made and the breathing became somewhat easier, but during the night, eight hours after admission to the hospital, the child died.

Pathology—Marked thickening of the mucous and submucous coats of the epiglottis, larynx and trachea, with a superficial necrosis that extended to the bifurcation of the trachea were found. The esophagus showed no pathologic changes, nor was there evidence of destruction of the mucosa of the mouth and pharynx.

4 Bose Indian M Gaz 40 139, 1905

5 Palmer Australian M Gaz 25 188, 1905

6 Ely, F A J A M A 54 1140 (April 2) 1910

CASE 6 (Reported by Watt⁷ in 1912) —A man, aged 63, took 1 ounce (30 cc) of solution of formaldehyd U S P (formalin 100 per cent) and died in two and one-half hours

Clinical Course—The patient left home about 8 00 a m without taking any breakfast He was seen about 9 15 a m, when near a well in a public park to become rapidly ill He staggered, doubled up over a low railing and retched viscid mucoid material He was put on a seat and soon was unconscious with his body stretched out and his head bent back over the seat The man was supposed by bystanders to be in a "fit," and was laid out on the grass with his collar loosened He was breathing "heavily," with chest heaving and eyes rolling, and was perspiring moderately Accounts differ about pallor or flushing of the face No convulsions or twitchings of the muscles were noticed nor was any attention drawn to coldness of the hands and face After being unconscious for about fifteen minutes, he gradually became conscious The

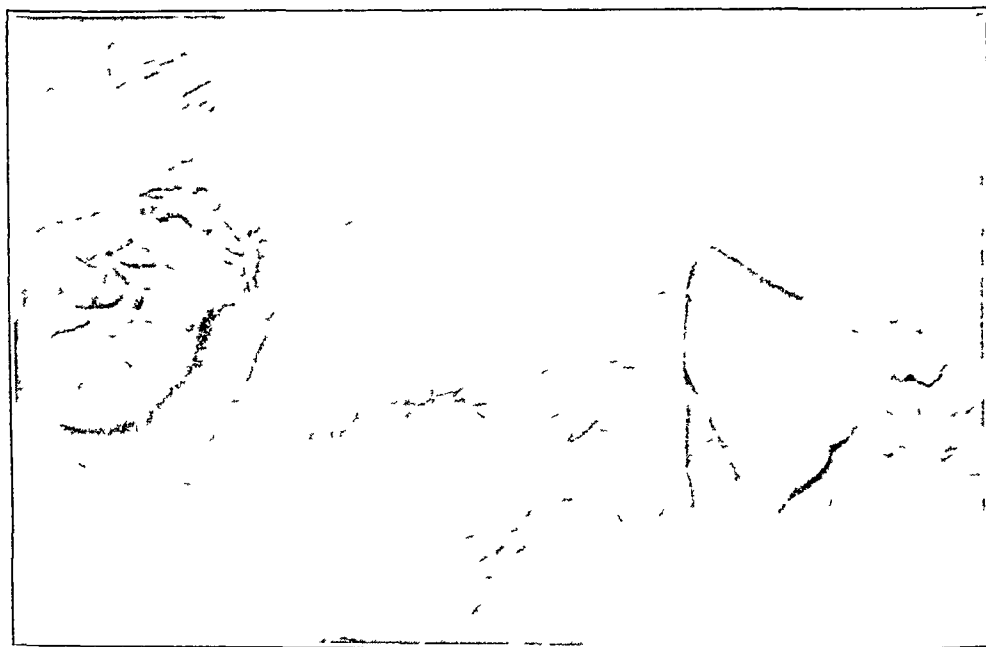


Fig 1—Esophagus, stomach and duodenum after formaldehyd poisoning of three hours' duration

man did not again vomit, but was now suffering very severe abdominal pain This caused him at intervals to double up almost on his hands and knees There was some froth about the mouth, but no discoloration of the lips or mouth was noted He staggered along with assistance for about 100 yards He then fell to the ground writhing with pain, and relapsed into unconsciousness, from which he did not again emerge

At 11 00 a m he was admitted to the hospital, unconscious, collapsed and almost pulseless The skin was pale, cold and clammy The mouth was opened with a gag but showed no discoloration and did not smell of formaldehyd All reflexes were absent on admission except the corneal reflex This also was lost in a few minutes, and respirations stopped The stomach was washed out, stimulants were given and artificial respiration applied, but death occurred at 11 53 a m

Pathology—No necropsy was done

CASE 7 (Reported by Moorhead⁸ in 1912) —A man, aged 30, took 3 ounces (89 cc) of solution of formaldehyd U S P, 10 per cent, and died in four weeks

Clinical Course—The patient was admitted to the hospital suffering from acute perforative appendicitis. An operation was performed immediately, and a gangrenous appendix was found along with diffuse suppurative peritonitis. Improvement after the operation was rapid and in a week the temperature had almost reached normal, though there was a profuse local discharge of pus. When in this condition, the patient went to a cupboard in the ward and drank the formaldehyd from a bottle there. About five minutes later, he was quite unconscious. The face was strongly flushed, the breathing stertorous, the pulse 96 to the minute, but steady and rather full. There was no discoloration of the lips or mouth, but there was a strong odor of formaldehyd from the breath. The pupils were dilated, conjunctival reflex was present, and the conjunctivae were injected. The skin over the chest and abdomen was pale and moist.

A stomach tube was passed and the stomach was washed out with strong ammonium acetate. The first washing smelled most strongly of formaldehyd, in fact overpoweringly so, and contained much altered blood. The washing was continued until no smell of formaldehyd could be detected from the water as it siphoned back from the stomach. Before this stage was reached, however, the patient recovered consciousness, in fact, he began to revive as soon as the first quantity of formaldehyd had been evacuated. He slept well that night and the next day he seemed almost completely recovered, except for some slight pain in the throat. No abdominal pain whatsoever was complained of. The first motions after the incident were fatty and contained a little mucus, but later though fluid, were free of blood.

The subsequent history was unfortunate, burrowing abscesses formed in the neighborhood of the appendix wound and general septicemia ensued. Death took place five weeks after admission.

Pathology—Patches of fat necrosis were found in the abdomen, the interior of the esophagus and stomach were extremely corroded, the most marked corrosion being found close to the pylorus. No signs of irritation were found in the duodenum or elsewhere in the alimentary canal. Apart from the changes commonly found in the organs in fatal cases of septicemia, nothing else abnormal was detected.

CASE 8 (Reported by Shaposhnikoff⁹ in 1912) —A woman, aged 21, took 30 cc of solution of formaldehyd U S P (formalin 100 per cent) and died in nine days.

Clinical Course—The patient, not knowing that she would become unconscious, lay without help until the next morning after she had taken the dose. She then had a severe burning in the abdomen and in the throat, she cried for help. She was brought to the hospital. In a short time, she had her stomach washed out. A strong odor of formaldehyd came from her mouth. On examination there was marked hyperemia around the uvula and, in a few places, small white areas without epithelium. Laryngoscopy showed widespread hyperemia. There was shortness of breath. The pulse was 126, the temperature, 38.5 C. Lungs and heart were negative. There were tracheal rhonchi. The pain in the abdomen decreased. The urine in output, color and odor was normal, there was no albumin. She was given soda water and milk. Three days after admission, she was fully conscious, the pulse was 120, the temperature, 39.5 C. Pneumonia was found in the left upper and lower lobes. The urine, 1,000 cc, contained albumin. On the fourth day, there was pneumonia on the other side. The temperature was 40.8 C, the sputum was

⁸ Moorhead Brit M J 2 1470, 1912

⁹ Shaposhnikoff Terap Obozr, Odessa, 5 537, 1912

gangrenous and had a putrid odor, the pulse was 130. Heart sounds were very weak, there were no murmurs. The amount of the urine was 800 cc. The patient was in marked stupor.

On the ninth day after admission, there was a marked putrid odor of the mouth. She could not be aroused, and died during the day in asphyxia.

Pathology—The diagnosis was diffuse bilateral bronchopneumonia. No report of the abdominal organs or esophagus was made.

CASE 9 (Observed by Christie¹⁰ in 1914)—A woman, aged 33, took an unknown quantity of solution of formaldehyd U S P (formalin percentage unknown), and died in nine and one-half hours.

Clinical Course—The patient was admitted shortly after drinking solution of formaldehyd (formalin percentage and quantity unknown). There was con-

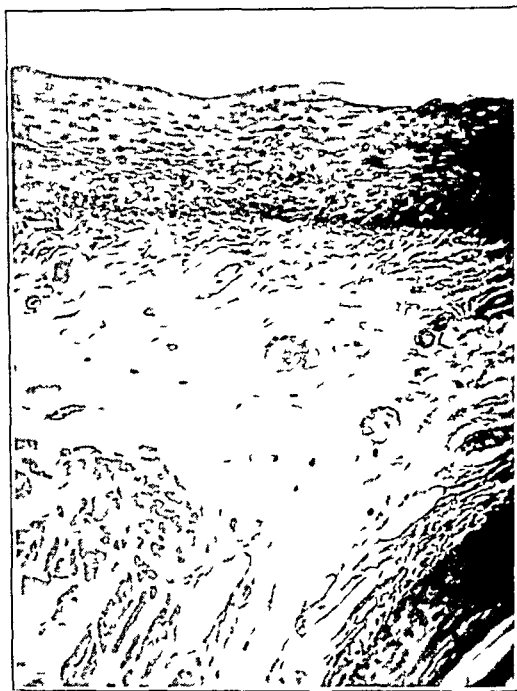


Fig 2—Edema of the submucosa and muscle of the esophagus after formaldehyd poisoning of three hours' duration.

siderable abdominal distress. The pulse was not obtainable at the wrists. The temperature rose steadily to 101.5 F just before death.

Three and one-half hours after admission, the patient was extremely nervous and flighty.

Fifteen minutes before death, no pulse was obtained at the wrists. There was extreme cyanosis and marked restlessness.

A heavy trace of albumin and granular casts were noted in the urine.

Pathology—No necropsy was done.

CASE 10 (Reported by DeRechter¹¹ in 1914)—A woman, aged (?), took 1½ ounces (45 cc) of solution of formaldehyd U S P (formalin 32 per cent) and died in between twenty and thirty minutes.

Clinical Course—The patient had immediate pain and vomited black, bloody masses, the pulse was feeble. She died in between twenty and thirty minutes.

¹⁰ Christie. Personal communication from the author. The patient died in Lakeside Hospital, Cleveland, in 1914.

¹¹ DeRechter. Arch Int de Med Legale 5 44, 1914.

Pathology—The hardening to a leathery consistency of the mucosa of the tongue, cheeks, esophagus, stomach and the uppermost meter of the small intestine was observed. There also was hardening of portions of organs neighboring the stomach (lung, spleen and pancreas).

CASE 11 (Reported by Marx¹² in 1919)—A woman, aged 27, took 4 ounces (118 cc) of solution of formaldehyd U S P (formalin 47 per cent) and died in sixty-two hours.

Clinical Course—The patient had pain in the chest and epigastrium, followed in fifteen minutes by vomiting. These symptoms continued for a few hours. The gastric lavage was followed by dyspnea, cyanosis of the face and hands and a feeble pulse. There was no loss of consciousness. There was no improvement of the pulse following stimulation.

No disturbance of micturition occurred. The temperature was normal for the first few days, rising to 100 F shortly before death.

Pathology—Marked hyperemia and edema of the brain and lungs and ecchymosis of the pleural epicardium were present, 200 cc of purulent exudate was observed in the abdominal cavity. No abnormalities of the mucosa of the

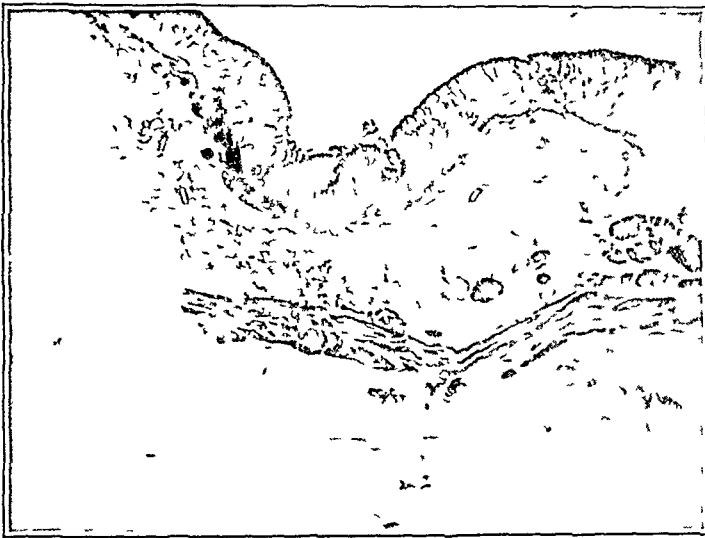


Fig 3—Early acute inflammation of stomach, showing linear ulcers, hemorrhage and edema, after formaldehyd poisoning of three hours' duration

lips, floor of the mouth or cheeks were seen. That of the pharynx and larynx was injected but intact. The esophageal mucosa was grayish red, dry and somewhat wrinkled, with changes most marked toward the stomach. The stomach, contracted and grayish-red, contained about 100 cc of brown matter with intermingled flecks of sloughed mucosa. The mucosa of the stomach was markedly tumefied and on the crests of the folds was converted into a friable eschar. In the region of the pylorus, the sloughs were white. These peeled off readily. The underlying areas were grayish red. In portions of the fundus, the mucosa in places was completely absent. The stomach wall was everywhere thickened and infiltrated by dark blood.

Microscopic Examination—Parenchymatous and fatty degeneration of the liver parenchyma was observed with degeneration of the kidneys and patchy necrosis of the tubal epithelium, especially in the region of the pyramids, with marked hyperemia. The heart muscle showed no abnormalities. In the lower portion of the esophagus in places, there was loss of surface epithelium with necrosis of the tunica propria in these areas. In other places the epithelium

was perfectly intact. The stomach showed marked alteration. The entire surface epithelium was necrotic. In many places the necrosis reached the muscularis mucosae. The submucosa showed extensive hemorrhage and small mononuclear round cell infiltration reaching to the serosa.

CASE 12 (author's case) —A woman, aged 33, took from 2½ to 3 ounces (74 to 89 c c) of solution of formaldehyd U S P (formalin 100 per cent) and died in three hours.

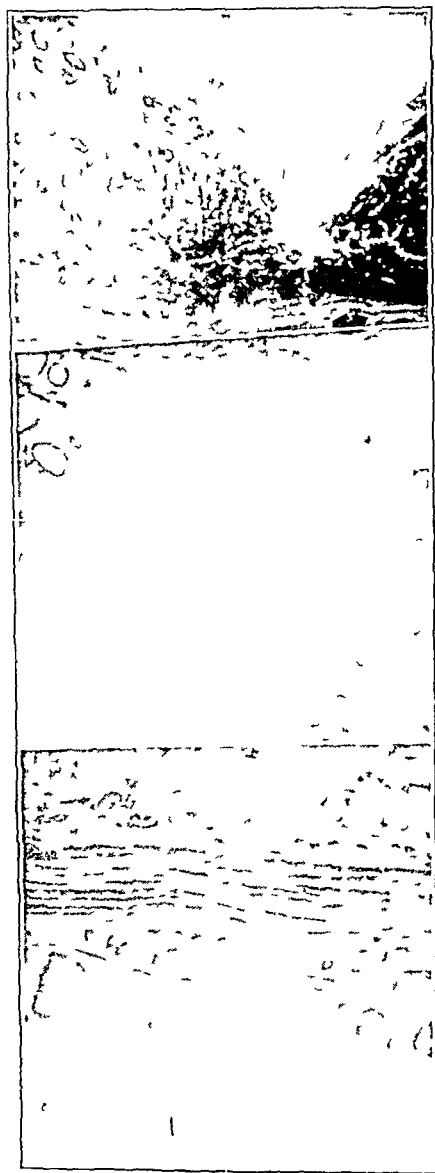


Fig 4—Linear ulceration, hemorrhage and marked edema of stomach after formaldehyd poisoning of three hours' duration

Clinical Course—The patient, who was unconscious, was brought to the hospital forty-five minutes after drinking the formaldehyd.

She was a slender, white woman, unconscious and in shock. Her pulse was thready, her eyes were half opened, the conjunctivae were injected, and the skin was cold and clammy. Gastric lavage was performed. Stimulants were given intravenously. The patient did not respond and died three hours after the ingestion of the formaldehyd.

Pathology—Necropsy was performed eighteen hours postmortem. The anatomic diagnosis was early acute gastritis (marked) following ingestion of formaldehyd solution, early acute enteritis (moderate), acute cardiac dilatation (light) moderate, terminal pulmonary edema (slight), and healed peritoneal adhesions. The probable cause of death was shock following formaldehyd poisoning with marked early acute gastritis and moderate early enteritis.

Of interest in the necropsy were the following points. There were a few hemorrhages observed in the mucous membrane of the inner surface of the lips. The mucosa of the esophagus throughout was whitish, perhaps slightly thickened. The submucosa showed some thickening by clear fluid. The stomach was several times the average size, contained about a liter of partially digested food and brownish, somewhat oily fluid, which on distillation gave the typical reaction for formaldehyd. The walls were greatly thickened, from 5 to 10 mm. This was most marked in the mucosa and submucosa, especially near the cardia, which was watery and gelatinous in appearance. The mucosa had a pigskin appearance with linear ulcerations and an almost diffuse dull reddish appearance suggesting hemorrhage. The duodenum showed moderate swelling, injection



Fig 5—Early inflammation with marked edema of duodenum after formaldehyd poisoning of three hours' duration

and hemorrhage of the mucosa. The cecum and ascending colon also showed some injection. The remainder of the colon and rectum showed no appreciable abnormalities.

Microscopic Description—Section of the esophagus showed an intact surface epithelium. The submucosa and to a less extent the muscle showed a moderate amount of amorphous pink stained material separating the fixed tissue. The blood vessels were engorged and contained somewhat more numerous nucleated cells than normal. There were a few wandering cells in the regional fixed tissue.

One section of the stomach showed in two places superficial ulceration of the mucosa a few hundred to 500 microns in width, extending only half way through the mucosa. About these ulcers there was marked infiltration of the tissues by red blood cells (not laked). In the base of each there were abundant wandering cells. The striking change in the mucosa was apparently marked edema. The mucosa was 2.5 mm in width. The blood vessels were everywhere engorged. There were areas of hemorrhage and a moderate number of wandering cells in the loose stroma. The hemorrhage about the ulcerations continued for a small distance into the submucosa. This coat was strikingly

edematous apparently, in places 4 mm in width. The muscle and serosa showed a similar picture of marked edema. The blood vessels were somewhat engorged. There were no wandering cells in the fixed tissue of the outer coats. A second section, from 7 to 8 mm in width, showed a similar picture with areas of linear ulceration, one of which continued to the submucosa. The blood in the mucosa was in striking contrast to that in the serosa. In the former position the red cells were intact, in the latter they were laked. Both sections showed striking engorgement of the vessels in the inner portion of the submucosa.

Section of the duodenum showed a picture of moderate edema of the mucosa and marked edema of the submucosa. The engorged blood vessels in the latter coat, with the blood in great part, was laked, and there were a moderate number of wandering cells in the mucosa and submucosa. The innermost portion of the mucosa, especially of the villi, was in great part necrotic, with considerable separation of the tissue by amorphous, pink stained material. The picture resembled postmortem change.

Section of the jejunum showed considerable desquamation of the surface epithelium. There was slight edema of the mucosa and apparently moderate edema of the submucosa. A number of blood vessels showed within and about them a moderate number of wandering cells. The blood in great part was laked.

SUMMARY OF PATHOLOGIC CHANGES IN FATAL CASES

Of the twelve fatal cases, ten came to necropsy. In all these, the changes were most marked in the lower esophagus and to an even greater extent in the stomach. The changes in these organs varied from simple hardening of the tissues to extreme corrosion. Frequently, marked congestion and edema were present with areas of erosion and of hemorrhage. Occasionally similar but less marked changes were observed in the duodenum and even jejunum. The remainder of the alimentary canal rarely showed any abnormality. In cases continuing thirteen hours or longer after ingestion of the formaldehyd, degenerative changes in the parenchymatous organs were noted, varying from slight cloudy swelling to fatty degeneration and even patchy necrosis. In a few cases the blood was still fluid and was dark red at the time of the necropsy. One case of sixty-two hours' duration showed 200 c c of purulent exudate in the abdominal cavity. In this case there was considerable involvement of the stomach with inflammatory changes reaching to the serosa. In one case death was apparently due to diffuse bronchopneumonia. In a few others, there was a terminal pulmonary edema. In one case the changes were practically limited to the respiratory tract as far as the bifurcation of the trachea.

CHANGES IN THE HEART RHYTHM ASSOCIATED WITH CHEYNE-STOKES RESPIRATION

DISPLACEMENT OF PACEMAKER TO BRANCHES OF
BUNDLE OF HIS *

WILLIAM H RESNIK, M D

AND

F W LATHROP, M D

BALTIMORE

The influence of respiration and of vagal stimulation on the location of the cardiac pacemaker and on the form of the ventricular electrocardiogram has been reviewed by Wilson,¹ and need not be detailed here. In brief, so far as man is concerned, the pacemaker may be displaced from its normal location at the sino-auricular node to the auriculo-ventricular node, and vagal stimulation may affect the form of the ventricular electrocardiogram by producing a bundle branch block. In the case described below, marked alterations in the cardiac mechanism were associated with Cheyne-Stokes respiration and were brought about by vagal stimulation. In this instance, changes in the form of the ventricular complexes were due to displacement of the pacemaker into the branches of the His bundle, and it is the purpose of this paper to report such a disturbance in the cardiac mechanism.

REPORT OF CASE

The patient, a colored man, aged 43, entered the hospital, Aug 4, 1924. His complaint was "shortness of breath and swelling of the legs." About three months before his admission, he began to suffer with dyspnea on exertion, cough, nausea, epigastric discomfort, and edema of the ankles and legs. These symptoms increased in severity, and he was obliged shortly to leave his work.

On physical examination, he presented the usual signs of severe myocardial failure. His heart was enlarged, and there were typical signs of dilatation of the aorta and aortic insufficiency. The blood Wassermann reaction was positive. The respiration was labored and Cheyne-Stokes in character, with periods of apnea of from twenty to twenty-five seconds' duration. At the end of a period of apnea, the heart rate slowed rapidly but not abruptly, to a rate considerably below that present during the period of dyspnea. About fifteen to twenty seconds after the onset of respiration, the heart rate began to increase and, within about twenty seconds, was again equal to that observed during the preceding period of dyspnea. No improvement took place with the usual forms of therapy, including digitalis administration. After the development of a cerebral vascular accident and a resultant hemiplegia, he was transferred, August 28 to another hospital, where he died a month later. Permission for necropsy was not obtained.

* From the Cardiographic Laboratory of the Johns Hopkins Hospital and University Medical Department.

1 Wilson, F N. Arch Int Med **16** 86 (July) 1915, *ibid*, **16** 1008 (Dec) 1915.

The relation between the changes in cardiac mechanism and respiration is shown in a semidiagrammatic chart (Fig 1), based on a continuous record of the heart and respiration taken during an entire apneic and part of the following dyspneic period

It may be stated at once that the various changes in cardiac mechanism described below were dependent on vagal stimulation. Suitable doses of atropin caused their complete disappearance.

Arterial Blood Gases—The peculiar and constant relationship of the onset of vagal stimulation to the end of the apneic and beginning of the dyspneic phases of respiration, and its persistence up to the height of the dyspneic period, suggested that the increase in vagal tone was brought on by the same factors that initiated respiration. Before the electrocardiographic curves are described, it may be of some interest to examine

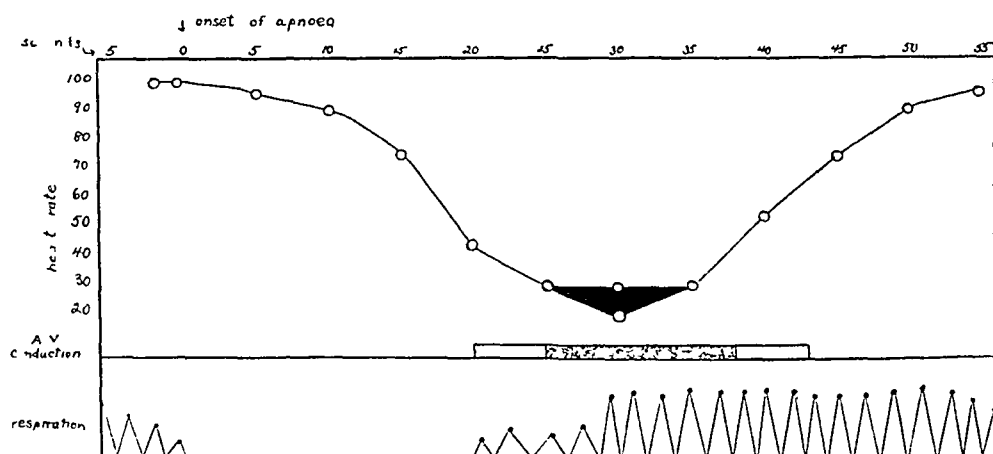


Fig 1—Semidiagrammatic chart based on continuous electrocardiographic record on which respiration was recorded. Upper curve, heart rate, accurate except at the lowest rates. Since these occurred during a relatively short period of time, any changes that occurred would be greatly exaggerated if calculations were made for the rate per minute. The shaded area shows the difference between ventricular and auricular rates, the ventricular rate at this time always being greater than the auricular which sometimes averaged only 4-8 beats per minute. Middle line, condition of auriculoventricular conduction, the straight line being the normal, the lightly stippled area representing slight block, and the heavily stippled area, more marked or complete block. The exact time at which these changes took place could not be determined, and this diagram is more or less schematic. The lower curve represents the respirations, and is accurately charted from the respiratory curve.

certain data, which, although incomplete, give some information concerning the cause of the periodic increases in vagal tone.

Arterial blood was obtained during both the apneic and dyspneic periods. The needle was inserted in the brachial artery and the collection of blood was started about five seconds before the end of apnea. With the first attempt at inspiration, the patient was requested to continue to hold his breath in expiration for five more seconds until a

satisfactory sample was obtained. The needle was retained in the artery until the height of dyspnea, when he was requested to continue breathing at the same rate and depth until a second sample of blood was procured. The specimens of blood may be considered to represent good averages of the arterial blood at the end of apnea and at the height of dyspnea. The blood was obtained under oil and handled with the usual precautions. It was analyzed in duplicate by the method of Van Slyke and Stadie.²

The figures obtained on analysis are shown in the accompanying table.

There was practically complete oxygen saturation during the dyspneic, and a high degree of saturation at the end of the apneic period. Opposite changes took place in the carbon dioxide content which was somewhat lower than the normal. These results confirm those previously obtained by analyses of the alveolar air at different periods of the respiratory cycle in Cheyne-Stokes respiration,³ during dyspnea, the tension of

Arterial Blood Gases

	End of Apnea (Duration, 20 Seconds)	Middyspnea
Oxygen content	15.78% by volume	16.15% by volume
Oxygen capacity	16.17% by volume	
Oxygen saturation	97%	100% *
Carbon dioxide content	41.06% by volume	38.87% by volume

* This figure is calculated on the basis of the oxygen capacity of the apneic blood. Since the blood during the dyspneic period was undoubtedly more alkaline,³ the oxygen capacity would have been higher, and the oxygen saturation slightly lower.

oxygen increases, the tension of carbon dioxide decreases, during apnea, reverse changes take place. In addition, it has been found that the hydrogen ion concentration of the blood is higher at the end of apnea than during dyspnea.⁴

According to Haldane,⁵ Cheyne-Stokes respiration is always an evidence of oxygen want. As the arterial blood was practically fully saturated in this patient, anoxemia must have been chiefly of the stagnant type, the slight anemia being disregarded. In the absence of analyses of the venous blood such an assumption is reasonable in view of the fact that the patient was suffering from a severe degree of heart failure.

It has been found⁴ that administration of oxygen or carbon dioxide causes the periodicity in Cheyne-Stokes respiration to disappear. In our patient, the addition of carbon dioxide in the presence of a high concen-

2 Van Slyke, D. D., and Stadie, W. C. *J. Biol. Chem.* **49**: 1 (Nov.) 1921.

3 Hale White, W., Ryffel, J. H., Poulton, E. P., Johnson, W., and Chisholm, R. A. *Quart. J. Med.* **7**: 389, 1913-1914.

4 Hale White et al. (Footnote 3). Pembrey, M. S. *J. Path. & Bacteriol.* **12**: 258, 1908.

5 Haldane, J. S. *Brit. M. J.* **1**: 409 (March) 1921.

tration of oxygen had such an effect⁶ As the increased concentration of oxygen in the inspired air could have had no appreciable effect in raising the arterial oxygen content, the disappearance of Cheyne-Stokes breathing was due to the increased stimulus to respiration brought about by elevation of carbon dioxide tension of the arterial blood

Since the administration of carbon dioxide caused the disappearance of the periodic increases of vagal tone, it seems probable that the occurrence of this phenomenon at the end of the apneic period was not due to the increased carbon dioxide tension and hydrogen ion concentration of the blood over that prevailing during the dyspneic period It appears more likely that the slight lowering of arterial oxygen saturation added to the already marked stagnant anoxemia produced a final anoxemia

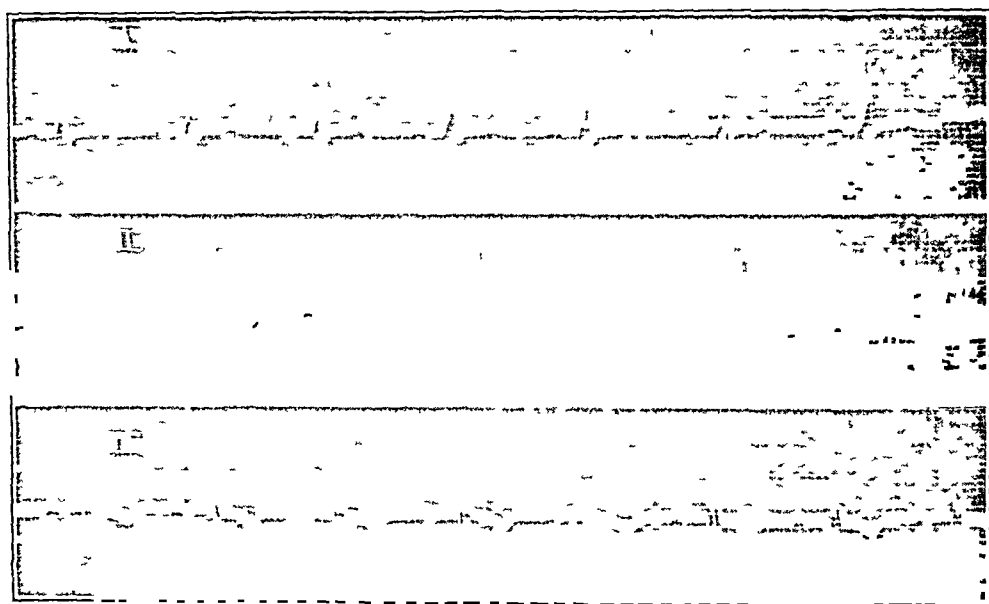


Fig 2—Normal and more rapid rhythm In this and subsequent figures, the heavy time lines represent 0.2 second

of sufficient severity to stimulate the cardio-inhibitory center⁷ That the heightened vagal tone persisted and increased during the early part of the dyspneic period is ascribed to the shallow and consequently ineffective respirations at this time

6 Actually, we attempted to administer oxygen without increasing the carbon dioxide content of the inspired gas This was done before we knew the figures of the blood gas analyses When it was learned that the dyspneic blood was practically fully saturated with oxygen and that oxygen administration could have had no significant effect in increasing the oxygen content of the arterial blood, we were led to examine the apparatus used for oxygen administration and found that its soda-lime was not effective in completely absorbing carbon dioxide, that is, the patient had been subjected to increasing tensions of carbon dioxide Owing to the critical condition of the patient we were unable to make any further respiratory studies

7 Greene, C W, and Gilbert N C Am J Physiol 60 155 (March) 1922

DESCRIPTION OF ELECTROCARDIOGRAPHIC CURVES

In Figure 2 are shown the electrocardiograms taken during the normal and more rapid rhythm. The rate was 100 or slightly less, the P-R and Q-R-S intervals were 0.20 and 0.08 second, respectively, the R waves were notched, the T waves were negative in Leads II and III. We shall refer to the type of complexes represented in this curve as the normal for the individual.

Displacement of Pacemaker to Bundle Branch—Figure 3 (Lead II), taken on August 6, after the patient had received 0.8 gm. of powdered digitalis leaf, illustrates the last three cycles of the rhythm prevailing during the period of slow heart action and the resumption of the normal rhythm. The R-R intervals of the three cycles are 2.25 and 2.22 seconds, respectively. No P waves can be seen to precede the ventricular complexes, which are of abnormal form. Following the T wave of the second cycle there is a small wave of auricular origin to which there is no ventricular response. The T wave of the third ventricular cycle is deformed by another P wave, which is followed after 0.36 second by

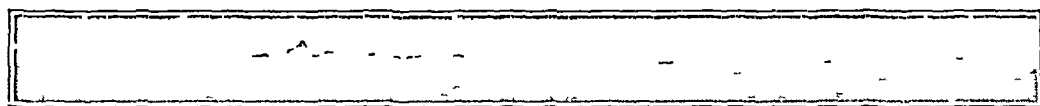


Fig 3—Lead II, showing termination of abnormal rhythm and onset of normal rhythm. No P waves precede the first three ventricular cycles, which are of abnormal form. Shortly after the second T wave and superimposed on the third T wave are the outlines of auricular waves.

a normal ventricular complex. This is succeeded by the restoration of the normal rhythm and a gradual increase in heart rate.

The gradual change in rate before the onset of the new rhythm (shown in other figures) and its slow rate show that it is of the so-called homogenetic type. There are two possible interpretations of this rhythm: (1) The impulse may have originated in the His bundle and have traversed one branch due to a blocking of the other, (2) the impulse may have arisen in one of the bundle branches of the ventricular conduction system.

Figure 4 (Lead II), taken on the same day, shows the changes in cardiac mechanism occurring at the end of the apneic and the beginning of the dyspneic phases of respiration. The heart rate, which six seconds previously had been about 85, is slowing rapidly. The first three cycles are of normal form. The third is followed after an interventricular interval of 2.04 seconds by a ventricular complex similar in form to those present at the beginning of Figure 3, and it is reasonable to assume that its origin is the same. It is preceded by an auricular wave of normal form which has no relationship to it, since the distinctly shortened P-R interval indicates that the auricular impulse arising at the sino-auricular

node has reached the ventricle after it had entered into activity. The fifth ventricular beat is an extrasystole. The sixth and seventh ventricular cycles (the last and first of the upper middle curves, respectively) are of normal form, and occur in response to supraventricular stimuli. The eighth ventricular cycle takes place after an interventricular interval of 2.30 seconds and its form is similar to the fourth. It is followed by an inverted P wave, and after a P-R interval of 0.21 second there is a normal ventricular complex. The succeeding cycles occur at gradually increasing rates and have a normal form. The relationship of these changes to respiration is well shown by the respiratory curves at the bottom of the records.

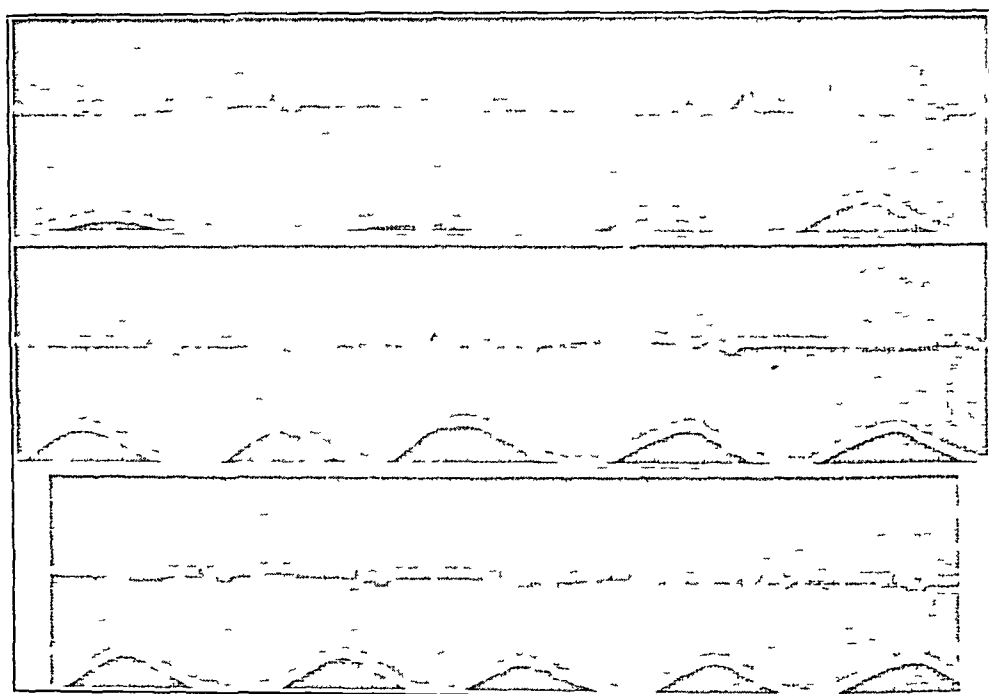


Fig 4—Lead II, showing onset of idioventricular rhythm. The curves are continuous, and illustrate the relationship of the changes in rhythm to respiration. The fourth and eighth ventricular complexes are abnormal in form, the sixth and seventh, normal, responding to auricular impulses. The ninth ventricular cycle occurs in response to a stimulus arising in the auriculo-ventricular node. Following this, the normal rhythm is restored. The curve at the bottom of the record represents respiration, the upstroke being inspiration. The records begin with the onset of the dyspneic period.

These curves demonstrate that, of the two possibilities for the origin of the abnormal rhythm, the second is the correct one, namely, the origin of the ventricular impulse was in one of the branches of the His bundle. When, after the onset of this rhythm, the auricular impulses occur at such a time that the ventricle can respond (Cycles 6 and 7), the form of the ventricular complex is normal. The possibility that a bundle branch block may have existed and disappeared is eliminated by the fact

that when the auricular impulses drop out again, the abnormal type of complex returns (Cycle 8)

Figure 5 (Lead I) is a curve taken, August 7, after the patient had been given 1 gm of powdered digitalis leaf. The respiration is not recorded, but, as was noted on all previous occasions, the slowing and change in rhythm occurred at the end of a period of apnea. The first two cycles are of normal outline. The third ventricular cycle is not preceded by an auricular wave, and its form is abnormal. A blocked auricular wave follows after an interval of 0.36 second. The next ventricular cycle is similar to the fourth. An auricular wave follows after an interval of 0.52 second, and is responded to after 0.23 second by a normal ventricular complex. The sixth and eighth ventricular cycles are similar in form to the third and fourth, and both are followed by ectopic beats. Between the last two pairs of ventricular beats can be seen the outlines of apparently normal auricular waves. They follow the preceding ventricular cycles at intervals of 0.28 and 0.3 second respectively. The occurrence of the normal type of ventricular complex in response to the preceding auricular impulse (fifth ventricular cycle)

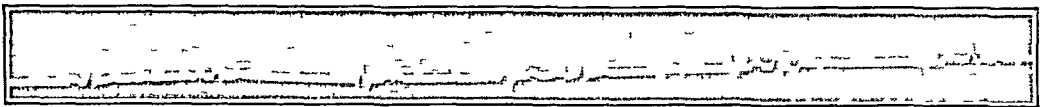


Fig 5—Lead I, showing onset of idioventricular rhythm at third cycle. The fifth ventricular complex is normal, responding to an auricular impulse. There are blocked auricular beats after the third, sixth and eighth ventricular cycles. The seventh and ninth ventricular cycles are extrasystoles.

soon after the onset of the abnormal rhythm points even more conclusively to the assumption that the normal form of the ventricular complex in this instance was not merely due to a temporary disappearance of vagal tone. The abnormal rhythm persisted for approximately twenty seconds after its onset, and it is almost certain that vagal tone was increasing rather than decreasing at the time when this ventricular complex was recorded.

For the same reason, the seventh and ninth ventricular beats probably have no relation to the auricular impulses which precede them, since the refractory period of the auriculoventricular node was greater than 0.36 second but a few seconds before at the onset of the heightened vagal tone.

Other records illustrating essentially the same events were procured on different occasions. Although the changes in rhythm were present before digitalis was given, it was found that after effective doses of digitalis, sufficient to produce nausea and vomiting, the abnormal rhythm began to appear earlier in the apneic periods, due undoubtedly to an increase in vagal tone by the drug.

The duration of the pause necessary for the development of the abnormal rhythm varied on different days. Thus, the intervals preceding the two idioventricular beats in Figure 4 were 2.04 and 2.30 seconds. In Figure 5, these intervals were 1.48, 1.42, 1.47 and 1.46 seconds. Rarely, the idioventricular rhythm failed to appear even after a pause greater than was usually necessary on that day. For example, in Figure 4, the interval between the eleventh and twelfth cycles, both normal, was 2.23 seconds, whereas the first idioventricular beat occurred after 2.04 seconds. It may be seen, in Figure 5, that the returning cycles of the idioventricular rhythm after disturbance by the normal cycle or the premature ventricular beats were of approximately the same length as the cycles of the idioventricular rhythm (comparison should be made with Figure 7, taken a few minutes later). This has been described before by Hering⁸

Auriculoventricular Rhythm—August 11, there was a short period of auriculoventricular rhythm, which began during a period of apnea

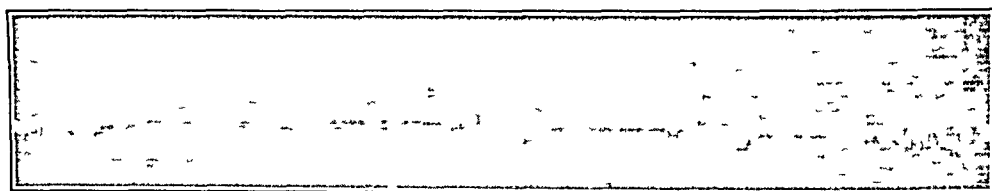


Fig. 6—Lead III, auriculoventricular rhythm

and persisted to the middle of the next dyspneic period. This rhythm is illustrated in Figure 6 (Lead III). The inverted P wave and the shortened P-R interval (0.13 second, the normal for the individual is 0.2 second) point to the upper part of the auriculoventricular node as the site of origin of the rhythm.

Auriculoventricular Block—The changes in cardiac mechanism described above were associated with auriculoventricular heart block (Fig. 1). In general, it was found that, with the development of the abnormal rhythm, the P-R interval of a normal interrupting cycle was increased. With the continuation of the idioventricular rhythm, auricular beats were blocked sometimes after a long ventricular pause, apparently adequate for recovery of the auriculoventricular node. The degree of auriculoventricular block diminished just before the restoration of the normal rhythm.

In Figure 5 there is a blocked auricular impulse occurring 0.36 second after the onset of the third ventricular cycle, 0.52 second after

⁸ Hering, H. E., cited by Lewis, T. *The Mechanism and Graphic Registration of the Heart Beat*, London, 1920.

the next ventricular cycle there is an auricular wave which is followed by a normal ventricular complex after a P-R interval of 0.23 second, the usual P-R interval for this subject being 0.20 second. Figure 7 (Lead I) is a continuation of a record in which the idioventricular rhythm had been present for about fifteen seconds. In the upper part of the curve are six cycles of the idioventricular rhythm. No definite P waves are seen. Thirty-two hundredths second before the seventh ventricular cycle (the first of the lower record which is a direct continuation of the upper) and 1.16 seconds after the sixth ventricular cycle (the last of the upper curve), there is a normal auricular wave. Since the ventricular cycle following the auricular wave is exactly similar in outline to the other ventricular complexes of the same rhythm, one may conclude that it bore no relation to the auricular impulse, and that there was present a complete auriculoventricular block, since, after a long period of recovery, the ventricle failed to respond to the auricular impulse. Because of the movement of the patient, it is difficult to deter-

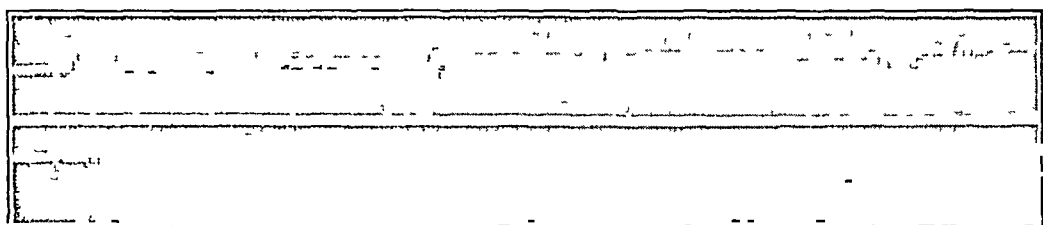


Fig 7—Lead I, showing idioventricular rhythm which had been present for fifteen seconds. The upper and lower curves are continuous. Between the sixth and seventh ventricular cycles (the last of upper curve and first of lower) there is a blocked auricular complex, occurring 1.16 seconds after the sixth complex. The tenth ventricular complex is normal, responding to an auricular impulse, the preceding R-R interval is 1.36 seconds. The next ventricular cycle is idioventricular in origin, the preceding R-R interval being 1.52 seconds. The twelfth and thirteenth ventricular cycles (the last two of the lower curve) are normal, and are the first two of the restored normal rhythm. The P-R interval of the twelfth cycle is 0.32 second, of the thirteenth 0.2 second.

mine whether or not an auricular beat occurs between the eighth and ninth ventricular cycles. The next auricular beat is followed by a supraventricular complex after a P-R interval of 0.22 second. Four-tenths second after the eleventh ventricular cycle there is another auricular wave, and, owing to the short period of recovery, the ventricle responds after an interval of 0.32 second. The P-R interval of the next (and the succeeding cycles of the restored normal rhythm) is 0.20 second, normal for this subject. In Figure 4, the ninth ventricular complex occurs in response to an inverted P wave after a P-R interval of 0.21 second. The form of the P wave points to the auriculoventricular node as the site of origin of the stimulus for this cycle. Since the P-R interval was increased over the normal interval of 0.20 second,

that is, vagal stimulation caused a forward block, the impulse must have arisen in the upper portion of the node⁹

Auricular Standstill—When the idioventricular rhythm persisted for from twenty to twenty-five seconds, impulse formation in the auricle was frequently inhibited to such an extent that only one auricular wave was seen in a period of about fifteen seconds

Comment on Changes in Rhythm—The changes in cardiac mechanism due to vagal stimulation that we have described above are consistent with previous observations, experimental and clinical. In this patient, the important effect of vagal stimulation was to produce displacement of the pacemaker to the branches of the His bundle, auriculoventricular block of varying degree, auriculoventricular rhythm, and marked slowing of impulse formation at the sino-auricular node. Experimentally, the development of heart block and of auriculoventricular rhythm, and the displacement of the pacemaker into one of the branches of the ventricular conduction system, have been described as a result of vagal stimulation¹⁰. In man, heart block due to vagal stimulation is well known. Wilson¹ has reported the appearance of auriculoventricular rhythm during deep respirations. So far as we can determine, no other instance in man has been described in which the pacemaker has been displaced to one of the divisions of the His bundle by vagal stimulation.

Often the idioventricular beat was followed after a short interval, usually 0.22 to 0.28 second, by an auricular wave. Such an occurrence is illustrated in Figure 5. The auricular waves were always of normal outline, indicating that their origin was in the sino-auricular node. The fairly frequent association of the idioventricular beat followed by an auricular contraction of normal origin permits the suggestion, previously made by Wilson and Robinson,¹¹ that the contraction of the ventricle may have stimulated the normal pacemaker mechanically.

SUMMARY AND CONCLUSIONS

In a patient with Cheyne-Stokes respiration, marked changes in cardiac mechanism occurred at the end of the apneic and the beginning of the dyspneic periods. These changes were displacement of the pacemaker to the auriculoventricular node and the bundle branches of the main stem of the conduction system, depression of auriculoventricular conduction and conspicuous slowing of impulse formation at the sino-auricular node. These changes were due to vagal stimulation.

⁹ Lewis, T. *Heart* 5:247, 1913-1914.

¹⁰ Wilson (Footnote 1, second reference). Cohn, A. E. *J. Exper. Med.* 18:715, 1913.

¹¹ Wilson, F. N., and Robinson, G. C. *Heart Block Arch. Int. Med.* 21:166 (Jan.) 1918.

THE OUTPUT OF THE HEART PER BEAT IN HEART DISEASE¹

I M RABINOWITCH, M D

MONTREAL

Heart failure, generally, has been recognized to result in slowing the circulation of the blood. The volume of blood passing through the body at any moment is numerically represented by the product of the pulse rate and the output per heart beat. Since the pulse rate is generally increased in cases of heart failure, it appears reasonable to infer that the slowing of the circulation is due to a greater relative decrease in the output per beat.

The output of the heart per minute in man has been measured by numerous workers¹. In normal subjects, it has been found to vary between 3.8 and 6 liters. Thus, Lowey and von Schrotters (1903) give 4.2 liters, Plesch (1909), 3.1-5.3, Markoff, Muller and Zuntz (1912), 4-6, Muller (1913), 4.5-5.5, Boinstein (1913), 5-6, Christianson, Douglas and Haldane (1914), 4, Means and Newburgh (1915), 4-4.5, Lundsgaard (1916), male 5.3, female 3.8, and Meakins, Dautrebande and Fetter (1923), male 6.5-8 and female 5.5-7 liters. Lundsgaard² found a marked decrease in the volume output of hearts in disease as compared with those of normal persons.

Usually one of two methods, or modifications of them with no essential change in the underlying principles, is employed in determining the output of blood per unit of time in man. One method is based on measurement of the amount of nitrous oxid taken up in unit time from a mixture of nitrous oxid and air with which the lungs are filled. The other consists of simultaneous determinations of the oxygen contents of arterial and venous blood, and the rate of oxygen absorption in the lungs.

In calculations based on data in which the latter procedure is employed, it is generally assumed that the arterial blood is saturated, or nearly so (95 per cent). Though this assumption may be approximately correct in many instances other than heart disease, in the latter this does not always hold true. It is generally recognized that during heart failure, owing to passive congestion of the lungs, there may be a marked impairment in the efficiency of the latter to aerate completely the blood.

¹ From the Department of Metabolism, Montreal General Hospital.

² This work was done with the technical assistance of Miss Kathleen Wylde.

1 Lundsgaard, C. J. Exper. Med. **27** 129 (Jan.) 1918, Deutsch. Arch. f. klin. Med. **118** 361, 1916. Henderson, Y. Physiol. Rev. **3** 165 (April) 1923.

2 Lundsgaard, C. Untersuchungen über das minuten volume des Herzens bei Menschen, Deutsch. Arch. f. klin. Med. **118** 513, 1916.

passing through. Usually, also, calculations for the venous oxygen are obtained indirectly, i. e., from the use of dissociation curves.

With the development of simplified technical procedures within recent years for the direct determination of the oxygen consumption by the tissues and the oxygen content of arterial and venous blood, no such assumption or indirect calculations are necessary. I have, therefore, attempted to determine the clinical utility of such procedure.

The object of this communication is to record the results that have been obtained in an effort to determine the values for the output of the heart per beat by a combined study of arterial and venous oxygen content and respiratory air analyses with laboratory methods presently available.

CRITICISM OF METHOD

Since variations in the oxygen content of the blood from an arm vein might be caused by factors other than the output of the heart per beat, the chief objection that may be made to conclusions drawn from such observations is that the blood is obtained from a single region (arm) and is assumed to represent the average of that found from the whole body. It is known that there are many factors that influence the oxygen content of arm vein blood. These have been previously discussed by Lundsgaard³. This author has aptly suggested comparison of findings with clinical observations and postponement of the conclusions until sufficient data has been accumulated. A few observations appear necessary here and will be made briefly. The fact that the oxygen content of venous blood obtained from an arm differs from that entering the pulmonary artery appears to be admitted by Barcroft and Nagahaski (1921), Peters, Baar and Rule (1921), Uyeno and Doi (1922), Meakins et al (1922 and 1923) and Stewart (1923). More recently Abbott and Dawson⁴ rather strongly emphasized the unreliability of accepting venous blood values so obtained. These authors state "We cannot find the oxygen content of venous blood by using arm vein blood, with changes of temperature in arm environment, this blood varies all the way from nearly completely reduced to nearly arterial in composition," and quote the observations of Meakins and Davies to substantiate their view.

It is thus obvious that from a consideration of the foregoing observations alone, the method employed here for the estimation of the circulation rate would yield results of doubtful clinical value. One fact needs to be mentioned here, the significance of which will be pointed out later. The oxygen content of blood taken from an arm vein may not be the same as the oxygen content of the mixed venous blood entering the

³ Lundsgaard, C. Studies of Oxygen in Venous Blood, *J. Exper. Med.* 27: 179 (Feb.) 1918.

⁴ Abbott, M. E., and Dawson, W. T. *Internat. Clinics* 4: 155 (Dec.) 1924.

pulmonary artery But, when taken under a uniform set of conditions in health, it appears to have a fairly constant composition For example, the oxygen unsaturation, when determined by the prescribed method, has been found to vary in persons with no heart failure within narrow limits (Table 1)

This is an important observation since because of this phenomenon values for the circulation may be deduced It is suggested that when the test is performed under the uniform set of conditions described, the value of the found circulation rate bears the same relation to the true circulation rate as the oxygen content of venous arm blood bears to the oxygen content of the mixed venous blood entering the lungs That is, it is possible to obtain a relative measure of the circulation rate Judging from the literature, it appears that by all other procedures values of only such nature are obtained This may be attributed chiefly to two causes, namely, variations due to (a) uncontrollable physiologic phenomena and (b) technical difficulties

TABLE 1—*Oxygen Unsaturation in Persons With no Heart Failure*

	Venous Oxygen Unsaturation
12 normal persons (Lundsgaard)	25 to 9 cc
12 patients with compensated heart lesions (Lundsgaard)	25 to 8 cc
40 observations on 4 patients with compensated heart lesions (Lundsgaard)	15 to 56 cc
55 observations on patients with anemia (Lundsgaard)	25 to 55 cc
10 normal persons (author's observations)	35 to 55 cc
15 normal persons (Harrop)	26 to 88 cc

In testing the clinical utility of a laboratory procedure, it need hardly be emphasized that a relative measure of a phenomenon has essentially the same practical value as an absolute measure For example, it is recognized that the methods generally employed for the estimation of blood sugars do not yield absolute but relative values, since they represent not only sugar but total reducing substances Day to day fluctuations noted in the course of the treatment of a diabetic patient, however, are of definite clinical significance, bearing a relation to total carbohydrate tolerance Numerous such examples may be cited

The values recorded here for the circulation rate are, therefore regarded as relative In an individual the absolute value for the circulation rate may be expressed as the product of the relative value and a constant Thus, $R = r \times k$, in which R = actual volume output per beat, r = calculated volume output per beat, and k = constant

In evaluating the clinical utility of any laboratory test, the clinical picture must be the guide It will be shown here that by performing the work under a definite set of conditions, there is correlation between the relative values of the circulation rate obtained and the clinical picture The differences found between the values in normal individuals

and those with heart failure are so marked and consistent that their significance appears suggestive, in spite of the possible disturbing factors referred to above

TECHNIC

The oxygen content and capacity of the blood were determined by the gasometric method of Van Slyke⁵ The technic for the collection of the venous blood from the arm vein was that followed by Lundsgaard⁶ Arterial blood was obtained from the radial artery following the procedure described by Stadie,⁷ that it is a procedure free from danger was first demonstrated by Hurter⁸ and since then by Stadie⁷ and Harrop⁹

In order to insure a successful radial puncture, it is essential to follow in detail the technic as described by Stadie⁷

An ordinary 20 c.c. all glass Luer syringe is used with a Luer needle 1 to 2 mm in diameter The point of the needle is beveled at an angle of about 45 degrees and must be very sharp To prevent the blood from coming in contact with air, 1 to 2 c.c. of sterile liquid petrolatum is poured into the barrel of the syringe, the plunger is inserted, and the syringe with the attached needle inverted The plunger is forced upward, and the air in the dead space at the distal end of the syringe and needle is expelled The excess of liquid petrolatum is then forced out so that only a small amount remains in the needle and in the small dead space The patient's arm is laid horizontally upon a pillow, the hand is flexed backwards, and the region over the radial artery is sterilized with tincture of iodine The end of the left index finger of the operator is then sterilized with iodine and, by using this finger in palpation, the best site for the puncture is determined (Since the position of the artery is determined by palpation, it is advantageous to avoid gloves and use the bare finger) The skin at the site of the proposed puncture is anesthetized with procaine The syringe and needle are held at an angle of about 45 degrees to the surface, the needle is then pushed through the skin, and, after carefully relocating its position, the needle is entered into the artery It is essential that the position of the artery should be sharply located and the point of maximum pulsation chosen (usually opposite the radial styloid), then the artery is easily entered, the pressure of the blood stream forces up the plunger so that suction is unnecessary, and within fifteen to sixty seconds from 10 to 20 c.c. of blood can be obtained The needle is then quickly withdrawn, and, by means of a compress, firm pressure is immediately applied over the artery for 1 to 2 minutes, so as to obliterate it temporarily and prevent extravasation The wrist is then bandaged with three or four thicknesses of compress to get greater pressure, and at the end of about three or four hours the bandage may be removed If the artery is missed at the first puncture, and especially if a hematoma begins to form or blood extravasates around the needle, the operator should desist at once

5 Van Slyke, D. D. Gasometric Determination of the Oxygen and Hemoglobin of Blood, *J Biol Chem* **33** 127 (Jan) 1918

6 Lundsgaard, C. Studies of Oxygen in the Venous Blood, *J Biol Chem* **33** 133 (Jan) 1918

7 Stadie, E. C. The Oxygen of the Arterial and Venous Blood in Pneumonia and Its Relation to Cyanosis, *J Exper Med* **30** 215 (Sept) 1919

8 Hurter. *Deutsch Arch f klin Med* **108** 1, 1912

9 Harrop, G. A., Jr. The Oxygen and Carbon Dioxide Content of Arterial and Venous Blood in Normal Individuals and in Patients with Anemia and Heart Disease, *J Exper Med* **30** 241, 1918

The blood thus collected is transferred to a tube 25 by 10 cm in which a layer of petrolatum at least 2 cm deep has been placed previously (to prevent contact with air) with some potassium oxalate to prevent coagulation. Not all cases are suitable for arterial punctures, especially when repeated punctures are contemplated. Many women and some men have small radial arteries, deeply situated, and hence difficult to puncture. The more rapid and bounding the pulse, the easier the puncture, and cases of pulse rates of 70 or below are difficult. Occasionally, when the artery has been touched with the point of the needle, it becomes pulseless, due, no doubt, to reflex vasoconstriction, but after fifteen to sixty seconds it relaxes and the puncture may be finished.

Following the puncture there is a numbness of the radial side of the hand, but this wears off quickly. About 50 per cent of the subjects complain of a very slight, dull pain at the wrist lasting about twelve to twenty-four hours, but this is without significance.

I have not met the possible complications, hemorrhage, thrombosis, embolism and aneurysm.

All observations were made in the postabsorptive state and after thirty minutes of complete muscular rest, *with the arm from which the blood was to be withdrawn at absolute rest for the same period of time*. The arterial blood was first obtained, and within a few minutes (from three to five) the venous blood was withdrawn. For the determination of the oxygen consumption, the Benedict-Roth basal metabolism apparatus was used. While the latter was in progress, the complete blood analyses were made.

The determinations essential for this particular study are (a) oxygen content of arterial blood, (b) oxygen content of venous blood, (c) oxygen requirement (cubic centimeters per minute), and (d) pulse rate.

From these are calculated (a) the oxygen consumption (that is the difference between arterial and venous oxygen content), (b) total output of blood per minute from the heart, and (c) the output per beat.

The formula for the relative output per beat is

$$T = \frac{100 \times r}{p(a - v)}$$

in which

T = the relative output per beat (cubic centimeters)
 r = oxygen requirement (cubic centimeters per minute)
 a = the arterial oxygen content
 v = venous oxygen content
 p = pulse rate

The combined data are recorded in Tables 2 and 3.

The clinical features in the cases of heart failure are, briefly, as follows.

CASE 1—A man, aged 53. Diagnosis: Chronic myocarditis, slight dyspnea at rest, no cyanosis. Vital capacity, 1,220 liters.

CASE 2—A man, aged 57. Diagnosis: Chronic myocarditis, auricular fibrillation, hydrothorax, chronic nephritis, semiorthopnea, general cyanosis. Vital capacity, 0,210 liters.

CASE 3—A man, aged 44 Diagnosis Malignant endocarditis, acute nephritis, marked, labored breathing in semiorthopneic position Vital capacity, 0 860 liters

CASE 4—A woman, aged 29 Diagnosis Chronic endocarditis, mitral stenosis, chronic nephritis, general anasarca, semiorthopnea, general cyanosis Vital capacity, 0 800 liters

CASE 5—A man, aged 62 Diagnosis Chronic myocarditis, chronic nephritis, marked, labored breathing in orthopneic position, marked general cyanosis Vital capacity, 0 155 liters

CASE 6—A man, aged 48 Diagnosis Chronic myocarditis, dyspnea at rest, no cyanosis Vital capacity, 0 510 liters

CASE 7—A man, aged 57 Diagnosis Chronic endocarditis, chronic myocarditis, general anasarca, labored breathing in orthopneic position, cyanosis of lips and fingers Vital capacity, 0 360 liters

CASE 8—A man, aged 61 Diagnosis Chronic endocarditis, mitral stenosis, right hydrothorax, labored breathing in orthopneic position, general cyanosis Vital capacity, 0 760 liters

CASE 9—A girl, aged 16 Diagnosis Acute rheumatic fever, chronic endocarditis, slight dyspnea at rest, no cyanosis Vital capacity, 1 120 liters

CASE 10—A man, aged 41 Diagnosis Malignant endocarditis, cardiac hypertrophy and dilatation, general anasarca, orthopnea, general marked cyanosis Vital capacity, 0 216 liters

In Table 2 are recorded the results obtained from ten persons regarded as normal so far as any evidence of heart disease was concerned These were, however, all hospital patients The significance of this fact is pointed out under the discussion of the results

In Table 3 are recorded the results obtained from ten patients with various heart lesions, all associated with heart failure

COMMENT ON RESULTS

The average relative ventricular output of blood per beat in ten normal persons at rest, and without any clinical signs of heart failure, was found to be 85 3 c c The maximum and minimum values noted were respectively 99 5 and 70 1 c c This differed strikingly from the results obtained from the cases of heart failure, in which the average output per beat was found to be 27 3 c c with a maximum of 46 2 and a minimum of 13 6 c c Thus the differences between the results obtained from normal persons and those suffering from heart failure are in themselves evidence of the significance of this data, in spite of the numerous disturbing factors to be considered in obtaining the blood for analysis from one particular region That the differences must still be somewhat greater than obtained in these cases is obvious from the fact that the subjects, though accepted as normal so far as heart disease was concerned, were all hospital patients

Because variations in the pulse rate may affect the ultimate result of such calculations, the data was recalculated on the assumption that in the normal persons the pulse rates were 10 points higher, and in the

patients with heart disease the pulse rates were 10 points lower than found. This would obviously result in decreasing the volume of the output per beat in the normal and increasing it in the pathologic cases.

The data so obtained is also recorded in the Tables 2 and 3, and marked differences are still noted between the results of normal and heart disease cases.

TABLE 2—*Output of Heart in Ten Patients with Heart Lesions*

Oxygen Capacity, C c	Arterial Oxygen Content, C c	Venous Oxygen Content, C c	Oxygen Consumption, C c	Arterial Oxygen Saturation, C c	Saturation of Blood, Per Cent	Venous Oxygen Saturation, C c	Cubic Centimeters Oxygen per Minute Requirement (Basal Metabolism)	Pulse	Output Per Minute	Output Per Beat	Output Per Beat with Pulse Plus 10 Points
162	157	107	50	05	97.0	55	257	68	5,140	75.5	65.9
182	174	140	34	08	95.7	42	224	74	6,588	89.0	78.4
153	149	118	31	04	97.4	35	201	80	6,483	81.0	72.0
174	167	130	37	07	96.0	44	257	76	6,916	91.3	80.7
168	160	123	37	08	95.3	45	217	74	5,864	79.2	69.8
154	145	108	37	09	91.2	46	220	68	5,945	87.4	76.4
149	142	110	32	07	95.4	39	241	82	7,531	91.8	81.8
169	160	131	29	09	94.7	38	189	74	6,517	88.0	77.5
158	148	110	38	10	93.7	48	192	72	5,052	70.1	61.6
161	152	117	35	09	91.5	44	244	70	6,971	99.5	87.1
Average					95.3					85.3	75.1

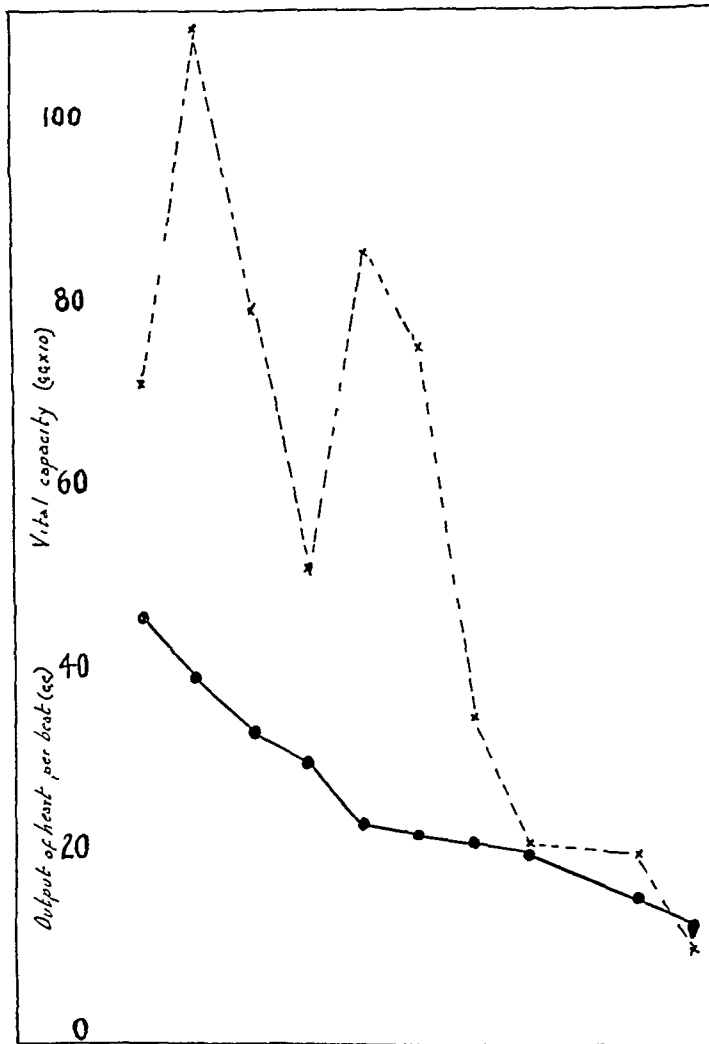
TABLE 3—*Output of Heart in Ten Patients With Heart Lesions*

Case	Oxygen Capacity, C c	Arterial Oxygen Content, C c	Venous Oxygen Content, C c	Oxygen Consumption, C c	Arterial Oxygen Saturation, C c	Saturation of Blood, Per Cent	Venous Oxygen Saturation, C c	Cubic Centimeters Oxygen per Minute Requirement (Basal Metabolism)	Pulse	Output Per Minute	Output Per Beat	Output Per Beat with Pulse Plus 10 Points
1	166	145	91	54	31	87.5	75	210	84	3,888	46.2	52.5
2	190	163	65	98	27	85.8	125	247	116	2,520	21.7	23.7
3	180	164	65	99	16	91.2	115	214	90	2,161	24.0	27.0
4	174	157	69	88	20	88.8	105	264	88	3,000	34.0	38.4
5	169	135	14	12.1	24	85.8	155	212	128	1,752	13.6	14.8
6	174	158	83	75	16	90.8	91	230	98	3,666	31.2	33.5
7	221	181	92	89	40	81.9	129	244	122	2,741	22.4	24.4
8	214	180	66	11.4	31	84.2	148	195	72	1,710	23.7	27.5
9	177	163	94	69	14	92.1	83	247	84	3,423	40.5	46.2
10	210	183	77	10.6	27	87.2	134	214	122	2,018	16.5	18.0
Average						88.5					27.3	30.6

From this data it would be unwise to formulate a definite relation between the output per beat and the clinical picture. Some general agreement was noted in that in Cases 5 and 10, the two apparently more ill patients, the relative values of the output per beat were found to be the lowest, and in two of these cases, Cases 1 and 9, in which there was no appreciable cyanosis and only a very slight dyspnea, the relative values of the output per beat were found to be the highest. There also appeared to be some general agreement in the values for the vital capacity. However this must be accepted with reserve. The vital

capacity data is recorded in terms of absolute values, since, in the majority of instances, the patients were bedridden and it was not possible to record them in terms of liters per square meter of body surface

The relation found between the total vital capacity and relative cardiac output is graphically recorded in the accompanying chart. The solid line represents the values for the cardiac output per beat. They are recorded from highest to lowest. The corresponding vital capacity in each is recorded by the broken line.



Relation between total vital capacity and cardiac output

In all cases of heart failure, with the exception of Cases 1, 6 and 9 there was evidence of cyanosis. An interesting feature is that in these three cases there was little or no evidence of impairment in the pulmonary exchange of gases. In Case 1 there was some impairment in the percussion note at the bases, but the breath sounds were heard. There were a few scattered moist râles. This, however, as Lundsgaard pointed out, is suggestive evidence that the alveoli were patent to air. In

Case 6, there was little impairment in the percussion note at the bases, but the breath sounds were heard. In Case 9, the lungs were normal throughout. When there was no appreciable lung involvement and we were able to exclude the possibility of the slowing of the circulation in the capillaries, due to vasomotor disturbances, it appeared interesting to correlate the degree of arterial oxygen saturation with heart function, i. e., output per beat. The arterial oxygen saturation values in these three cases were, respectively, 87.5, 90.8 and 92.1 per cent, all below the accepted average value for normal subjects, 95 per cent. There is thus suggestive evidence that slowing of the circulation alone may increase the arterial oxygen unsaturation. This applies particularly to Case 9, in which the lungs were found normal throughout.

SUMMARY

- 1 The relative output of the heart per beat was calculated from the results obtained by the direct determination of the oxygen content of arterial and venous blood in ten normal persons and in ten patients suffering from various heart lesions, associated with heart failure.

- 2 Marked and consistent differences were noted between the values in the normal and pathologic cases in all instances.

- 3 There appeared to be some general agreement between the clinical picture and the laboratory data.

- 4 The technical procedure is practical for clinical purposes and the results are of sufficient interest to warrant further observations along the same lines, especially since at present there is no quantitative method for the estimation of the efficiency of the heart.

DISEASES OF THE LIVER

I A SURVEY OF TESTS FOR HEPATIC FUNCTION[†]

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Progress in the science of medicine is dependent on the development of knowledge concerning anatomic and physiologic conditions in health and the disturbances in disease. Each advance, whether in the field of pathologic anatomy or of pathologic physiology, has made possible a better understanding qualitatively and quantitatively of the abnormal process, thus permitting more accurate diagnosis, more exact prognosis, and more rational therapeutics.

As details of the mechanisms controlling normal physiologic activity have come to light, attention has been more directly focused on the changes produced in these mechanisms by disease, and in recent years functional changes have been sought with as great, if not greater, interest than anatomic changes. Exact criteria regarding disturbances of function are not readily delimited. In too many conditions morbid anatomy, valuable as it unquestionably is, has been the only index available to physiologic changes.

Recent improvements in biochemical methods, and consequently in our knowledge of chemical physiology, have been of great practical value, especially in the study of the parenchymatous organs which are the main chemical laboratories of the body. Particularly striking have been the advances in methods of determining changes in renal function. Experience in the comparative study of the different tests proposed has amply demonstrated the relative value of each. Still other tests that will give more accurate or convenient indexes to renal function may yet be devised, but at present many clinicians rely chiefly on the study of the phenol-sulphonaphthalein excretion and the urea and creatinin content of the blood.

The kidney is preponderantly an excretory organ, and consequently peculiarly suitable for functional study. Both the initial material, the blood, and the excretory product, the urine, are readily available for

[†] Read before the Society of Clinical Surgery, Rochester, Minn., June 7, 1924.

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analysis Furthermore, differences in the behavior of the organ toward various materials undoubtedly do exist, but they are differences largely of degree rather than of kind The functional study of the liver, on the other hand, is much more difficult Normally, the liver is actively concerned in intermediary metabolism The formation and excretion of the bile may be, and probably is, an entirely separate and distinct process, also a host of physiologic activities have been ascribed to the liver that may or may not be confined to that organ

Under such conditions, it is not surprising that a large number of tests have been devised as clinical indexes to functional changes in the liver Rowntree, Marshall and Chesney¹ in 1914 reviewed the tests in use at that time, and discarded many of them as of little or no clinical value We have attempted a combined experimental and clinical study of what appears to be the most promising tests in use at the present time, in order to determine their relative clinical value Many of the previously described tests have been purposely omitted from this discussion Particular attention has been paid to those furnishing quantitative data, in an effort to obtain evidence relating to the degree of hepatic damage We shall summarize here the theoretical basis, technic and present status of the various tests employed in this work The results obtained in a series of control cases are shown in the accompanying table Detailed reports will later be made with regard to the changes observed in a series of 800 cases comprising various pathologic states

CARBOHYDRATE METABOLISM

The importance of the metabolic activity of the liver, especially in carbohydrate metabolism, was first recognized following the discovery by Claude Bernard of the glycogenic function Evidences of disturbance in the glycogenic activity of the liver have long been sought Particular attention has been paid to the study of changes in carbohydrate tolerance, and the earlier studies concerning the relation of the liver to glycosuria have been summarized by Rowntree, Hurwitz and Bloomfield² Of the various sugars, glucose and galactose have proved unsatisfactory in clinical use, fructose has been more satisfactory, but the clinical importance of the fructose tolerance test is still undecided

1 Rowntree, L G, Marshall, E K, Jr, and Chesney, A M Studies in Liver Function, *J A M A* **63** 1533-1537 (Oct 31) 1914, Studies in Liver Function, *Tr A Am Phys* **29** 586-625, 1914

2 Rowntree, L G, Hurwitz, S H, and Bloomfield, A L An Experimental and Clinical Study of the Value of Phenoltetrachlorphthalein as a Test for Hepatic Function, *Bull Johns Hopkins Hosp* **24** 327-342, 1913, Der Wert des Phenoltetrachlorphthalein für die Funktionsprüfung der Leber, *Arch f Verdauungskr* **19** 751-753, 1913

Fructose Tolerance —It is outside the scope of this paper to attempt to correlate all the facts regarding the absorption, utilization and storage of fructose in the body. The theory, correct or otherwise, on which most observers agree and on which the use of this sugar as a test for hepatic function is founded, is briefly as follows. Fructose is rapidly absorbed from the digestive tract in the normal subject, and is very easily formed into glycogen by the liver. During the whole process, no marked increase in the blood sugar occurs. The rise of the respiratory quotient may be taken to indicate the combustion of a certain part of it. In cases of disease of the liver, glycogen formation supposedly is diminished, and the subsequent rise of blood sugar is assumed to be due in part to fructose that has not been stored or utilized. At least high blood sugar curves after fructose ingestion have been interpreted as due to failure of the glycogenic function of the liver.

Strauss³ in 1901 investigated hepatic function by an estimation of fructose tolerance. This was suggested by the work of Sachs on hepatectomized frogs. Strauss gave 100 gm of this sugar and examined the urine by the Seliwanoff reaction. In 90 per cent of a series of cases of hepatic disease, he obtained fructosuria, which he considered indicative of the inability of the liver to form glycogen from this material, and therefore a measure of hepatic insufficiency. In 10 per cent of his cases of disease of the liver, negative results were obtained, and in several normals, fructosuria occurred. He explained the negative results by assuming a compensatory hypertrophy of undamaged liver cells, and considered the positive results in normal persons as due to potential or obscure hepatic disorders. Churchman⁴ obtained about 24 per cent of positives in normal persons, and negative results in several patients with malignant disease of the liver. Bloomfield and Hurwitz⁵ likewise failed to find the test of clinical value, they objected to it on theoretic grounds, asserting that hypophyseal, pancreatic and neurogenic disorders might alter carbohydrate tolerance, that other organs or even undamaged hepatic tissue might compensate in cases of disease of the liver to give a normal test, and that variations in the renal threshold for fructose must be considered if renal disease was present. Practically, they found that such large doses of sugar caused nausea and vomiting, besides giving

3 Strauss, H. Zur Funktionsprüfung der Leber, Deutsch med Wchnschr **27** 757-759, 1901. Sachs, Hans. Ueber Bedeutung der Leber für die Verwerthung der verschiedenen Zuckerarten im Organismus, Ztschr f klin Med **38** 87-126, 1899.

4 Churchman, J. W. The Strauss Test for Hepatic Insufficiency, Bull Johns Hopkins Hosp **23** 10-15, 1912.

5 Bloomfield, A. L., and Hurwitz, S. H. Tests for Hepatic Function, Clinical Use of the Carbohydrates, Bull Johns Hopkins Hosp **24** 375-379, 1913.

inconstant clinical results Folin and Berglund⁶ have recently emphasized the importance of impurities in the fructose in producing gastro-intestinal irritation and glycosuria Friedman⁷ concluded that, while fructose tolerance tests might give some information, they could not be interpreted in an absolutely specific manner Rowntree, Hurwitz and Bloomfield,² and Rowntree, Marshall and Chesney¹ in their studies on hepatic function finally discarded the Strauss test as of no clinical value

With the advent of satisfactory methods for the chemical analysis of the blood, the blood sugar curve, after the administration of carbohydrates, was studied as an index to the carbohydrate tolerance In 1913, Schirokauer⁸ reported blood sugar values one hour after the ingestion of fructose He found that in the normal subjects only the slightest rise was obtained, in distinction to the sharp increase noted after the taking of a large amount of glucose In hepatic disease, however, the increase in blood sugar was marked Bergmark⁹ later confirmed this work, and found that fructose caused only the slightest rise in the blood sugar of normal subjects Isaac¹⁰ asserted that fructose caused no appreciable rise in the blood sugar of normal subjects, although its presence could be demonstrated in the blood, and that in hepatic disease it formed a considerably larger fraction of the total blood sugar

The present interest in fructose tolerance is largely due to the work of MacLean and de Wesselow¹¹ They noted that fructose caused only a slight rise (less than 20 mg) in the blood sugar of normal subjects, and in 1920 began its use as a test for hepatic function Using MacLean and de Wesselow's methods, Spence and Brett¹² reported positive results in a variety of hepatic disorders They gave from 30 to 50 gm of sugar, and estimated the blood sugar every thirty minutes for two hours, a rise of 20 mg or more was considered positive They discarded fructosuria as a criterion of hepatic function, considering that

6 Folin, O, and Berglund, H Some New Observations and Interpretations with Reference to Transportation, Retention and Excretion of Carbohydrates, *J Biol Chem* **51** 213-273 (March) 1922

7 Friedman, J C, and Strouse, Solomon The Nonspecificity of Carbohydrate Tolerance Tests, *Arch Int Med* **14** 531-535 (Oct) 1914

8 Schirokauer, Hans Zur Funktionsprüfung der Leber, Die alimentäre Lavulose-Hyperglykämie, *Ztschr f klin Med* **78** 462-475, 1913

9 Bergmark, G Zuckerresorption und Blutzuckerspiegel, *Jahrb f Kinderh* **80** 373-385, 1914

10 Isaac, S Theoretisches und Klinisches zur Stellung der Lavulose im Stoffwechsel, *Med Klin* **2** 1207-1210, 1920

11 MacLean, H, and de Wesselow, O L V The Estimation of Sugar Tolerance, *Quart J Med* **14** 103-119 (Jan) 1921

12 Spence, J C, and Brett, P C The Use of Levulose as a Test for Hepatic Inefficiency, *Lancet* **2** 1362-1366 (Dec 31) 1921

the renal threshold for this sugar might show wide variations. Covell¹³ in 1923, using the same dosage of fructose and the Folin-Wu technic for estimating blood sugar, obtained positive results in a variety of hepatic disorders, including such tropical diseases as sprue and amebic dysentery. Using the same criterion, he obtained positive results in several cases of amebiasis and assumed that hepatic insufficiency was a common finding. Tallerman,¹⁴ using a dose of from 30 to 50 gm of fructose in proportion to body weight and MacLean's method of estimating blood sugar, made a careful study of the test, he concluded that variations in normal subjects were greater than generally appreciated, and that unless the height of the curve was 135 mg per cent or more, with a maximal rise of at least 30 mg, the finding should be considered negative.

Modern experimental work on animals with these recent modifications of the fructose test has been done by Bodansky.¹⁵ Using 3 gm of sugar for each kilogram of body weight as a standard dose, he estimated the normal tolerance of dogs for glucose, fructose and galactose. The animals were given the sugar dissolved in 200 c c of water by stomach tube, after an initial estimation of their blood sugar when fasting. Blood specimens were then taken after fifteen, forty-five, seventy-five and one hundred thirty-five minutes. Fructose produced the least rise in the curve, galactose the most, glucose occupying an intermediary position. There was no rise greater than 35 mg in normal animals after the administration of fructose. In dogs who had been under prolonged chloroform anesthesia, a sharp drop in fructose tolerance was found, due presumably to injury of the liver. In other animals, similar changes occurred after intramuscular injections of chloroform in oil. Actual hepatic damage was proved by necropsy. In animals that recovered, the fructose tolerance improved on regeneration of the liver. Similar results were obtained with other hepatic poisons, such as phosphorus and hydrazin and its derivatives.

Method of Determining the Fructose Tolerance—Patients were given a constant dose of 40 gm of fructose dissolved in 200 c c of lemonade after an initial blood sugar estimation. All patients had fasted for fourteen hours before the test and took no food until it was completed.

13 Covell, G. The Levulose Tolerance Test for Hepatic Efficiency, and Its Application in Certain Tropical Diseases, *Guy's Hosp Rep* **73** 354-367, 1923.

14 Tallerman, K. H. The Levulose Test for Liver Efficiency, and an Investigation of the Hepatic Condition in Pregnancy, *Quart J Med* **17** 37-52 (Oct) 1923.

15 Bodansky, M. Fructose, Glucose, Galactose Tolerance in Dogs, *J Biol Chem* **56** 387-393 (June) 1923, The Effect of Chloroform and Phosphorus Poisoning on Carbohydrate Tolerance, *ibid* **58** 515-522 (Dec) 1923, The Action of Hydrazin and Some of Its Derivatives in Producing Liver Injury as Measured by the Effect on Levulose Tolerance, *ibid* **58** 799-811 (Jan) 1924.

Blood samples were taken after one and two hours and the blood sugar estimated according to the method of Folin and Wu. The changes in blood sugar in normal persons have been variable, although no greater rise than 30 mg was noted. We have therefore followed Tallerman,¹⁴ whose criterion for a positive test is a maximal rise of 30 mg or more, with a one or two hour blood sugar percentage of 135 mg or more. Detailed reports of our studies in various pathologic conditions will be reported later, but in general we find that the difficulty of excluding extrahepatic influences, especially pancreatic disease, militates against the clinical usefulness of the test as a specific index to changes in the functional activity of the liver.

The Bodansky technic was used for dogs, the animals being given 3 gm of fructose for each kilogram of body weight, dissolved in from 200 to 300 cc of water, by stomach tube. All animals fasted fourteen hours or more. Blood specimens were taken before the sugar was administered and fifteen minutes, one hour and two hours afterward. Accepting Bodansky's criterion, we considered a test positive which showed a rise of 35 mg or more above the level in the initial specimen. In neither human beings nor dogs was the finding of glycosuria considered significant, in view of the known unreliability of this factor. Because the lesion produced experimentally is much more specific than that observed clinically, a correspondingly greater degree of reliance may be placed on changes in the fructose tolerance.

Blood Sugar Level—Mann and his associates, in consequence of their experiments on hepatectomized dogs, have further emphasized the rôle of the liver in maintaining the normal blood sugar level. Following operation there is a gradual and progressive fall in the blood sugar, when it decreases below a certain critical level, the body cannot function normally and death occurs. As this critical level is approached a characteristic group of symptoms develop which culminate in the so-called hypoglycemic reaction, first described by Mann¹⁶ and observed clinically in connection with insulin treatment. Hypoglycemia alone can be readily and successfully combated by the administration of glucose, but while this measure will prolong the life of the hepatectomized animal, it must be remembered that total abolition of the normal functional activity of the liver is incompatible with life. Fian and Isaac¹⁷ and, later, Williamson and Mann¹⁸ have shown that hypoglycemia may occur as

16 Mann, F C, and Magath, T B. Studies on the Physiology of the Liver, II, The Effect of the Removal of the Liver on the Blood Sugar Level, *Arch Int Med* **30** 73-84 (July) 1922.

17 Frank, E, and Isaac, S. Ueber des Wesen des gestorten Stoffwechsels bei der Phosphorvergiftung, *Arch f exper Path u Pharmakol* **64** 274-292, 1911.

18 Williamson, C S, and Mann, F C. Studies on the Physiology of the Liver, V, The Hepatic Factor in Chloroform and Phosphorus Poisoning, *Am J Physiol* **65** 267-276 (July) 1923.

a terminal event in phosphorus or chloroform poisoning. Opie and Alford¹⁹ showed the effect of a diet rich in carbohydrates in reducing the toxic action of these poisons. Previous experimental work has indicated that hypoglycemia develops only with practically complete abolition of the glycogenic activity of the liver. Consequently, this condition is not to be expected in the usual types of hepatic disease.

PROTEIN METABOLISM

The importance of the liver in protein metabolism is also recognized, but its exact rôle is not so well understood as it is in carbohydrate metabolism. The topic has been discussed in detail by Van Slyke²⁰ and Folin and Berglund,²¹ and will not be repeated here. Mann²² has carefully studied changes after ablation of the liver. He finds, as in the earlier studies on animals with Eck fistulas, that there is an increase in the excretion of ammonia and in the proportion of undetermined nitrogen in the urine. In the blood he finds a gradual decrease in the urea, and the rapid appearance of a substance giving the colorimetric reactions of uric acid, of which only traces normally occur in dog's blood. The creatin-creatinin metabolism is not affected. The amino-acids and the nonprotein nitrogen in the blood may be increased slightly, but the changes are much less striking than those in the urea or the uric acid fraction. There have been numerous clinical studies dealing with the nitrogen partition of the urine in patients with hepatic disease, which have arisen from the earlier identification of leucin and tyrosin crystals in the urine of patients with acute yellow atrophy of the liver. The previous work has been summarized by Rowntree, Marshall and Chesney,¹ and the study of experimental phosphorus and chloroform poisoning by Marshall and Rowntree.²³ These authors report the occurrence of increased ammonia and amino-acids and a decreased urea percentage in the urine. While definite changes were found and are undoubtedly of great significance from the physiologic point of view, the low level of protein metabolism so often present, together with the non-

19 Opie, E. L., and Alford, L. B. The Influence of Diet upon Necrosis Caused by Hepatic and Renal Poisons, I, Diet and the Hepatic Lesions of Chloroform, Phosphorus or Alcohol, *J. Exper. Med.* **21** 1-20, 1915.

20 Van Slyke, D. D. The Present Significance of the Amino-Acids in Physiology and Pathology, *Arch. Int. Med.* **19** 56-78 (Jan.) 1917.

21 Folin, O., and Berglund, H. The Retention and Distribution of Amino-Acids with Especial Reference to the Urea Formation, *J. Biol. Chem.* **51** 395 (April) 1922.

22 Bollman, J. L., Mann, F. C., and Magath, T. B. Studies on the Physiology of the Liver, VIII, Effect of Total Removal of the Liver on the Formation of Urea, *Am. J. Physiol.* **69** 371-392 (July) 1924.

23 Marshall, E. K., Jr., and Rowntree, L. G. Studies in Liver and Kidney Function in Experimental Phosphorus and Chloroform Poisoning, *J. Exper. Med.* **22** 333-346, 1915.

exclusion of acidosis, led them to the conclusion that the interpretation of the nitrogen distribution in the urine is somewhat difficult. The inconsistency of results and the further uncertainty regarding renal factors has led us also to question the clinical importance of a study of the nitrogen distribution in the urine as an index to changes in the physiologic activity of the liver.

Attention was later focused entirely on the changes in the blood. Rowntree, Marshall and Chesney¹ apparently were the first to make an extensive study of the nitrogen partition in the blood in its relation to hepatic disturbances. They found no evidence of nitrogen retention except in patients with renal insufficiency. While the changes were not constant, a moderate proportion of their cases showed a lowering in the percentage of the urea. They regarded a low percentage of urea nitrogen as compared to the total nonprotein nitrogen as suggestive of hepatic insufficiency. Our findings have been similar in character, although the changes noted have not been sufficiently well marked to have great diagnostic significance in individual cases.

In contrast to these observations are the experiments of Marshall and Rowntree²³ on the effects of phosphorus and chloroform poisoning. They found an increase in the nitrogenous products of the blood which they interpreted as evidence of an increased protein catabolism. They pointed out that the interpretation of their results was complicated by renal insufficiency which frequently appeared as a terminal phenomenon. Feigl and Luce²⁴ and Stadie and Van Slyke²⁵ have reported increases in the nonprotein nitrogen, blood urea, and amino-nitrogen of the blood in patients with acute yellow atrophy of the liver, while Stander²⁶ has observed similar changes in a fatal case of chloroform poisoning. As pointed out by Greene, Sandiford and Ross,²⁷ autolytic decomposition of the liver in the latter condition may be largely responsible for the increases noted. Other observers, such as Yanagi,²⁸ have suggested that the blood urea is decreased in patients with jaundice, while Gilbert,

24 Feigl, J., and Luce, H. Neue Untersuchungen über akute gelber Leber-atrophie, I, Ueber den Reststickstoff des Blutes und seine Komponenten, Weitere Beiträge zur vergleichenden Pathologie des Amino-saurenspiegels im Blute, *Biochem Ztschr* **79** 162-201, 1917.

25 Stadie, W. C., and Van Slyke, D. D. The Effect of Acute Yellow Atrophy on Metabolism and on the Composition of the Liver, *Arch Int Med* **25** 693-704 (June) 1920.

26 Stander, H. J. A Chemical Study of a Case of Chloroform Poisoning, *Bull Johns Hopkins Hosp* **35** 46-49 (Feb) 1924.

27 Greene, C. H., Sandiford, Kathleen, and Ross, Helen. The Amino-Acid Content of the Blood in Normal and Pathologic Conditions, *J Biol Chem* **58** 845-857 (Jan) 1924.

28 Yanagi, K. A New Colorimetric Method for the Determination of Urea with Urease, *J A M A* **82** 1169-1171 (April 12) 1924.

Chabrol and Benard²⁹ have reported an increase in the blood urea in patients with hemolytic jaundice

Mann³⁰ also has stressed the appearance in the blood of hepatectomized dogs of a substance giving the color reactions of uric acid. This substance appears regularly following removal of the liver, and he considers that in dogs the appearance of uric acid in the blood is very suggestive of decreased activity of the liver. This phenomenon has not been observed in man. The course of purin metabolism differs in man and dog, and it is quite possible that in this field of metabolism especially a direct transference of results from one species to the other should be made with reserve.

BILE PIGMENTS

Since the most striking hepatic disorders are those associated with icterus, it is natural that much attention should have been paid to the presence of bile pigments in the blood, urine and feces. Nature has fortunately provided an excellent index of disturbed pigment metabolism in the color changes in the skin and sclerae, and in the excretion of pigments in the urine and stools. The formation and excretion of bile are characteristic activities of the liver. It is generally accepted that the bile salts are formed by the hepatic epithelium, but it is becoming increasingly apparent that bile pigments may be formed largely outside the liver, although excreted by that organ. Furthermore, as has been repeatedly demonstrated, bile retention rapidly damages the parenchyma of the liver.

A century ago, Tiedemann and Gmelin,³¹ in an historically noteworthy book, reported their studies on the physiology of digestion. They described the color changes produced by the oxidation of the bile pigments in acid solution, the series of colors produced by nitric acid still being known as Gmelin's test. By means of this test they demonstrated the presence of bile pigment in the blood of a dog following experimental ligation of the common bile duct, and shortly afterward they successfully applied this test to the serum from a patient with jaundice. It is recognized that, while other allied pigments may occasionally be found in the blood, bilirubin is the only bile pigment present in the serum in appreciable amounts.

Icterus Index—Various attempts have been made to estimate the bilirubin content of the serum. The simplest methods are based on the assumption that bilirubin is the chief pigment of the serum and, accord-

29 Gilbert, A., Chabrol, E., and Benard, H. L'azotémie dans les ictères par hyperhémolyse, *Paris med* **35** 385-391 (May 8) 1920.

30 Bollman, J. L., Mann, F. C., and Magath, T. B. Studies on the Physiology of the Liver, X, Uric Acid Following Total Removal of the Liver, *Am J Physiol* **72** 629-646 (May) 1925.

31 Tiedemann, F., and Gmelin, L. *Die Verdauung nach Versuchen*, Heidelberg and Leipzig, K. Groos, pp. 1826-1827.

ingly, that the intensity of the serum color is an accurate index to the quantity of bilirubin. The serum color has been determined by measuring the number of dilutions necessary to render it just perceptible when viewed in a unit layer of serum, 1 cm thick. A colorimetric comparison, using a 1:10,000 solution of potassium bichromate as a standard, has also been used. The icterus index, based on the latter standard, has been tested clinically by Blankenhorn,³² Meulengracht,³³ Stetten,³⁴ Bernheim³⁵ and others. In general, they found that the values for normal serum are below 5. The threshold for clinical icterus lies between 8 and 15, while marked icterus is present in all cases with values above 30.

The icterus index may be used satisfactorily in following changes in the degree of jaundice in patients with frank icterus. In such patients bilirubin is the preponderate coloring matter, and changes in the serum color may reasonably be assumed to indicate variations in the amount of bile pigment. Conditions are different in specimens of serum in which the bilirubin content is little, if any, increased over the normal. Any cloudiness of the serum or the slightest trace of hemolysis interferes by changing both the quality and intensity of the color of the serum. Van den Bergh and Snapper³⁶ and Palmer³⁷ have studied the composition of the serum pigments, and find that serum from human beings contains xanthophyll and carotinoid pigments, which, although varying widely in amount, may be responsible for an appreciable portion of the serum color of the normal. We have observed two patients with pronounced discoloration of the skin and carotinemia after the prolonged use of carrots in their diet. The icterus index in one of the cases was 18, but by chemical methods the serum bilirubin measured only 0.5 mg per cent, an entirely normal amount. In the second case the icterus index was 26, but the serum bilirubin was only 1.1 mg per cent.

32 Blankenhorn, M. A. Acholuric Jaundice, *Arch. Int. Med.* **27** 131-134 (Jan.) 1921.

33 Meulengracht, E. Ein bilirubinkolorimeter behufs klinischer Bestimmung der Bilirubinmenge im Blute, *Deutsch. Arch. f. klin. Med.* **137** 38 (Aug.) 1921, abstr. *J. A. M. A.* **77** 1141 (Oct. 1) 1921.

34 Stetten, D. The Surgical Value of the Estimation of the Bile Pigmentation (Icterus Index) of the Blood Serum, *Ann. Surg.* **76** 191-200 (Aug.) 1922.

35 Bernheim, Alice R. The Icterus Index (a Quantitative Estimation of Bilirubinemia), *An Aid in Diagnosis and Prognosis*, *J. A. M. A.* **82** 291-295 (Jan. 26) 1924.

36 Van den Bergh, A. A. H., and Snapper, J. Die Farbstoffe des Blutserums, *Deutsch. Arch. f. klin. Med.* **110** 540-561, 1913.

37 Palmer, L. S. Carotinoids and Related Pigments, the Chromolipoids. New York, Chemical Catalogue Company, 1922.

The determination with certainty of changes in the amount of serum bilirubin necessitates the use of a method that will permit of the chemical separation or identification of the pigment. Many methods for the colorimetric determination of bilirubin have been suggested. By modifications in the principle of the original Gmelin test, the attempt has been made to secure green or blue oxidation products of bilirubin under conditions permitting quantitative estimation of the amount of color developed. The details of these various methods have been discussed by Gilbert,³⁸ Posselt,³⁹ van den Bergh,⁴⁰ Fouchet,⁴¹ and others. None of these methods has been wholly satisfactory, however. The rose colored azobilirubin has been a more satisfactory pigment derivative. The chemistry of the coupling of bilirubin with diazonium salts was investigated by Ehrlich,⁴² Proscher⁴³ and Orndorff and Teeple.⁴⁴ It remained for van den Bergh⁴⁵ to apply this reaction to the examination of blood serum, and to develop the test that bears his name.

Van den Bergh Test—Ehrlich found that when sulphanilic acid and sodium nitrite were added to solutions of bilirubin, a colored addition product, azobilirubin, was formed. This reaction is specific and will detect bilirubin in a dilution of 1:1,500,000. Van den Bergh precipitated the serum proteins with alcohol, which also serves as a solvent for bilirubin, and demonstrated that Ehrlich's reaction could be applied to this alcoholic extract. By comparison with solutions of pure bilirubin, a quantitative colorimetric estimation could be made.

Using this method, van den Bergh showed that bilirubin was a constant constituent of normal blood serum, and that the amount was much increased in cases of obstructive jaundice and other hepatic disorders. Later, on investigating the difference in reaction between bilirubin and solutions of human bile, he discovered that the latter gave

38 Gilbert, A., Herscher, M., and Posternak, S. Presentation d'un appareil pour doser la bilirubine dans le serum sanguin (cholemimetre), *Compt rend Soc de biol* **51** 700, 1904.

39 Posselt, A. Zur Methodik der klinischen Serumuntersuchungen Ueber den nachweiskleinster Gallenfarbstoffmengen im Blutserum (Fruhdiagnose des Ikterus), *Zentralbl f inn Med* **28** 489-509, 1907.

40 Van den Bergh, A. A. H. (with Snapper, J., and Muller, J.) Der Gallenfarbstoff im Blute, Leiden, S. C. Van Doesburgh, **8** 111, 1918.

41 Fouchet, A. Methode nouvelle de recherche et de dosage des pigments biliaires dans le serum sanguin, *Compt rend Soc de biol* **80** 826-828, 1917.

42 Ehrlich, Paul. Sulfodiazobenzol, ein Reagens auf Bilirubin, *Centralbl f klin Med* **4** 721-723, 1883.

43 Proscher, F. Ueber Acetophenonazobilirubin, *Ztschr f physiol Chem* **29** 411-415, 1900.

44 Orndorff, W. R., and Teeple, J. E. On Bilirubin, the Red Coloring Matter of the Bile, *Am Chem J* **33** 215-250, 1905.

45 Van den Bergh, A. A. H. La recherche de la bilirubine dans le plasma sanguin par la methode de la reaction diazoique, *Presse med* **29** 441-443 (June 4) 1921, abstr *J A M A* **77** 235 (July 16) 1921.

positive diazo reactions without the addition of alcohol. From this finding he concluded that there were two types of reaction, one obtained immediately after the addition of the diazo reagent, a "direct reaction," and the other after the addition of the diazo reagent and alcohol, an "indirect reaction." This was explained by the theory that the bilirubin in the bile was somewhat different in composition from the chemically pure substance.

Clinical observations with the van den Bergh reaction have been made by numerous observers (van den Bergh, Lepehne,⁴⁶ Rosenthal and Holzer,⁴⁷ McNee,⁴⁸ Andrewes⁴⁹ and others⁵⁰). It is now fully established that normal blood serum may contain from 0.5 to 2 mg per cent of bilirubin. Van den Bergh⁵¹ noted that certain apparently healthy persons have somewhat greater amounts of serum bilirubin than others, the former are said to have a physiologic hyperbilirubinemia. Gilbert described the condition, in 1905, as "simple familial cholemia."

In cases of obstructive jaundice, the direct reaction is always present. The amount of serum bilirubin may rise to as much as from 40 to 50 mg for each hundred cubic centimeters, but the amount of serum bilirubin does not necessarily parallel the discoloration of the skin or the amount of bile pigments in the urine. Mann and Bollman⁵² have shown that the van den Bergh reaction becomes indirect, biphasic and then direct in experimental animals following ligation of the common duct. Removal of the gallbladder at the time of ligation of the common duct hastens the onset of the direct reaction. A renal threshold for serum bilirubin in obstructive jaundice has been shown by van den Bergh, Lepehne and McNee.

46 Lepehne, G. Weitere Untersuchungen über Gallenfarbstoff im Blutserum des Menschen, *Deutsch Arch f klin Med* **135** 79-107 (Jan.) 1921, abstr *J A M A* **76** 1051-1052 (April 9) 1921, Pathogenese des Ikterus, *Ergebn d inn Med u Kinderh* **20** 221-280, 1921.

47 Rosenthal, F, and Holzer, P. Beiträge zur Lehre von den mechanischen und dynamischen Ikterusformen. Ueber die quantitativen Beziehungen von Bilirubin und Cholesterin im Blut bei den verschiedenen Ikterusformen, *Deutsch Arch f klin Med* **135** 257-280 (March) 1921.

48 McNee, J W. Jaundice. A Review of Recent Work, *Quart J Med* **16** 390-420 (July) 1923.

49 Andrewes, C H. The Nature of the Difference Between the Bilirubins of Obstructive and Haemolytic Jaundice, *Brit J Exper Path* **5** 213-219 (Aug.) 1924.

50 Feigl, J, and Querner, E. Bilirubinämie in ihren physiologisch-chemischen Beziehungen mit besonderer Berücksichtigung der diagnostischen Bedeutung, *Ztschr f d ges exper Med* **9** 153, 1919. Andrewes (Footnote 49).

51 Van den Bergh (Footnotes 40 and 45).

52 Mann, F C, and Bollman, J L. The Relation of the Gallbladder to the Development of Jaundice Following Obstruction of the Common Bile Duct, *J Lab & Clin Med* **10** 540-543, 1925.

Functional Tests in Control Cases Without Hepatic Disease

Case	Date	Age, Years	Sex	Urine			Blood Count			Blood Nitrogen Partition Mg per Cent				Bile Pigments (Serum)		Fructose Tolerance, Blood Sugar, Mg, per Cent			Phenol-tetra- chlorophthalein Dye in Serum, per Cent			Diagnosis							
				Specific Gravity	Albumin +	Casts +	Hemoglobin, per Cent	Erythrocytes Millions	Leukoocytes	Phenolsulphone Cent	Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen	Bile Index	Serum Bilirubin, Mg, per Cent	Van den Berg's Direct Reaction	1 Hour	2 Hours	Change		15 Minutes	1 Hour	2 Hours	Dye in Urine, Mg			
1	6/17/24	29	♂	1.018	0	0	68	3.81	5,800				29	26	2.6	1.5	5.1	17	2.0	0	88	107	100	19	9	3	0	0.0	Chronic nervous exhaustion secondary anemia
2	2/23/24	38	♂	1.010	0	0	78	4.53	8,800	90			30	38	3.2	1.4		4	0.3	0	98	100	100	2	9	2	0	0.2	Migraine
3	1/7/24	38	♂	1.028	0	0	76	4.55	6,200	50			29	38	2.4	1.7	6.0	6	1.0	0	94	106	100	12	7	2	0	0.0	Functional achylia
4	5/2/24	54	♂	1.029	0	0	81	4.80	7,800	30			27	27	2.4	1.7	6.1	8	0.9	0	90	115	83	25	7	2	0	0.0	Psychoneurosis, constipation
5	7/17/24	49	♂	1.032	0	0	80	4.49	6,800	65			29	35	2.9	1.7	6.1	8	1.3	0	89	106	94	17	4	3	0	0.4	Normal
6	2/6/24	26	♂	1.012	0	0	74	4.83	7,800				27	22	3.3	1.3		9	0.5	0	100	92	98	8	4	1	0	0.0	Psychoneurosis
7	1/4/24	56	♂	1.010	1	0	76	4.23	6,000				27	22	3.3	1.3		9	0.7	0	100	92	98	8	4	1	0	0.0	Chronic nervous exhaustion
8	10/10/23	34	♂	1.020	0	0	75	4.06	8,500	60			22	45					0.8	0	105	128	104	23	4	0	0	0.0	Normal
9	11/25/23	51	♂	1.024	0	0	76	4.06	7,400				22	18					0.8	0	94	100	90	6	3	0	0	0.0	Migraine
10	10/23/23	47	♂	1.015	0	0	79	4.06	9,200	40			26	18					0.4	0	70	85	85	15	2	0	0	0.0	Chronic appendicitis
11	1/3/24	25	♂	1.027	1	0	80	4.69	9,100				26	18	2.4	1.5	5.6	5	0.5	0	84	106	97	22	1	0	0	0.7	Chronic appendicitis
12	9/2/24	39	♂	1.010	0	0	70	4.69	9,100				26	18		2.4			0.5	0	84	106	97	22	1	0	0	0.0	Asthma
13	9/3/24	14	♂	1.025	0	0	70	4.48	10,300	65			31	21		2.4	6.0	7	0.9	0	100	117	116	17	10	3	1	0.3	Chronic infectious arthritis
14	6/4/24	26	♂	1.016	0	0	78	4.85	6,900	55			28	27	2.3	1.4	7.1	9	1.1	0	83	110	110	27	5	3	0	0.1	Chronic infectious arthritis
15	6/4/24	47	♂	1.026	0	0	77	4.90	16,400	55			34	27	2.3	1.4	7.1	9	1.1	0	83	110	110	27	5	3	0	0.5	Chronic infectious arthritis
16	6/1/23	52	♂	1.019	0	0	82	5.00	7,800				26	34	2.8	1.5	5.9				101	121	111	10	4	2	0	0.0	Chronic infectious arthritis
17	1/9/24	49	♂	1.022	0	0	60	3.76	7,800	55			26	26	1.9	1.4	6.1	5	0.5	0	111	85	108	-26	3	1	0	0.0	Chronic infectious arthritis, secondary anemia
18	4/29/23	39	♂	1.011	0	0	60	3.76	7,200				26	26							111	85	108	-26	3	1	0	0.0	Chronic infectious arthritis, secondary anemia
19	11/30/23	49	♂	1.019	0	0	60	4.20	8,500	50			21								3	1	0	3	1	0	0	0.0	Chronic infectious arthritis secondary anemia
20	8/7/24	26	♂	1.019	1	0	8	4.20	5,800	60			25	11	2.0	1.1	4.8		0.3	0	73	100	100	27	3	1	0	0.2	Chronic infectious arthritis, secondary anemia

* In this table, ♂ indicates male ♀ female

† Graded on a basis of 0 to 4

Although fairly large amounts of serum bilirubin may be present, in cases of hemolytic jaundice it is so masked that the direct reaction is not usually obtained, it is also believed that in this condition serum bilirubin does not pass the renal filter, even when the amount present is considerable. The indirect reaction has aided in the identification of latent jaundice in pernicious anemia, dibothriocephalic anemias and toxic icterus resulting from the administration of arsphenamin. The blood of new-born infants shows an increased serum bilirubin, and in certain instances high figures are said to presage the onset of icterus neonatorum.

In the toxic, catarrhal and infectious types of jaundice, the direct van den Bergh reaction almost invariably occurs, although it may be delayed. The figures for serum bilirubin obtained by the indirect reaction are usually high.

Van den Bergh cites a good deal of evidence derived from experimental and clinical material in support of his view that the direct reaction is a differential point between obstructive and nonobstructive jaundice, and this point has been particularly stressed by his followers, McNee and others. The experiments of Mann, Bollmann and Magath⁵³ showing the development of icterus in dogs following removal of the liver cast doubt on this view. In such dogs the serum gives first the indirect, then the diphasic, and finally a delayed reaction, which theoretically should not occur in a purely hematogenous icterus such as this. While the exact significance of the direct and indirect reactions is not yet entirely clear and the value of the direct reaction in differential diagnosis is doubtful, enough is known of the indirect reaction as a quantitative measure of the serum bilirubin to assure its accuracy and reliability for clinical purposes. Our study was confined to the amount of bilirubin in the serum indicated by the quantitative indirect reaction. In experimentally produced obstructive jaundice, a very close parallelism exists between the serum bilirubin and phenoltetrachlorophthalein retention, a fact that indicates the close relationship between the degree of icterus and hepatic efficiency.

Method of Determination of Bilirubin—The quantitative determination of the amount of bilirubin in the blood serum has been made by a modification of the indirect van den Bergh reaction, according to the method of Thannhauser and Anderson,⁵⁴ as follows. To 2 c.c. of

53 Bollman, J. L., Mann, F. C., and Magath, T. B. Studies on the Physiology of the Liver, IX, The Formation of Bile Pigment After Total Removal of the Liver, *Am J Physiol* **69** 393-409 (July) 1924.

54 Thannhauser, J. S., and Anderson, E. Methodik der quantitativen Bilirubinbestimmung im menschlichen Serum, *Deutsch Arch f klin Med* **137** 179-186 (Aug) 1921, abstr, *J A M A* **77** 1292 (Oct 15) 1921.

serum is added 1 c c of the freshly prepared sulphanilic reagent⁵⁵ The resulting color change permits the reading of the test in terms of the direct or indirect reaction Then 2 c c of a saturated solution of ammonium sulphate is added to help precipitate the serum proteins, followed by 10 c c of 96 per cent ethyl alcohol This produces a prompt intensification of the color corresponding to the indirect reaction of van den Bergh The contents of the tube are then centrifugalized and the clear supernatant fluid compared colorimetrically with an ethereal solution of ferric sulphocyanid, prepared according to the directions of van den Bergh⁵⁶ The color quality of the two solutions is not always identical, and other artificial color standards have been advised In general, however, the foregoing technic has been quite satisfactory

In their original paper, van den Bergh and Snapper reported their analyses in terms of the dilution of pure bilirubin required to give a solution of the same color concentration as the blood specimen Later, they⁴⁰ reported their results in terms of units, a 1 200,000 solution of bilirubin serving as a standard We have preferred to calculate the results in terms of the number of milligrams of bilirubin for each hundred cubic centimeters of solution, 1 mg per cent being equivalent to 2 units Because of the difficulty of securing pure bilirubin we have accepted van den Bergh's statement that the color of the ferric sulphocyanid standard is equivalent to a 1 200,000 solution of bilirubin, or 0.5 mg per cent All results obtained by the use of this standard are comparable, and the changes observed are so great that any minor error in the standardization of this solution may be ignored

55 This consists of two solutions, each of which keeps indefinitely, but the mixture must be made immediately prior to the test The two solutions are made up as follows

Solution A		Solution B	
Sulphanilic acid	5 gm	Sodium nitrite	0.5 gm
Concentrated hydrochloric acid	50 c c	Distilled water	100 c c
Distilled water	1,000 c c		

The diazo reagent consists of a mixture of these two solutions in the proportion of 25 c c of Solution A to 0.75 c c of Solution B

56 The artificial standard solution (van den Bergh) consists of iron sulphocyanid dissolved in ether The solution is made up of a color corresponding with a solution of azobilirubin of 1 200,000 The standard solution is made up as follows 0.1508 gm of ammonium iron-alum is dissolved in 50 c c of concentrated hydrochloric acid, and enough water added to make 100 c c This gives a solution of 1 320 normal, which keeps indefinitely To 10 c c of this solution is added 25 c c of concentrated hydrochloric acid and sufficient water to make 250 c c This gives a dilution of 1 8,000 normal, which keeps for about six months To 3 c c of this solution is added an equal volume of 20 per cent potassium sulphocyanid solution and 12 c c of ether After being well shaken and after all the reddish color has passed into the ether, the latter is carefully transferred into the colorimeter or other comparative tube This solution is in a concentration of 1 32,000 normal and must be prepared freshly each day that a test is made It corresponds in color, as has been indicated, to a 1 200,000 solution of azobilirubin, or 0.5 mg for each 100 c c

BILE SALTS

The present day evidence, although incomplete, is all in favor of the view that the bile salts are formed in the hepatic parenchyma. This conclusion is largely the result of studies of the biliary output of these substances, a field recently reviewed and extended in the publications of Whipple and his collaborators⁵⁷

In the clinical study of hepatic disease, much interest has centered around the possibilities of dissociated jaundice and variations in bile salt metabolism independent of changes in the bile pigments. Much of this work has been based on variations in the surface tension of the urine as shown by Hay's test, which is not specific in nature and does not permit of quantitative study. Foster and Hooper⁵⁸ devised an accurate method for the quantitative determination of bile acid excreted in the bile, but this method is not applicable to the smaller amount of bile acids in the urine and blood. Many investigators, among the more recent, Tashiro,⁵⁹ have attempted to devise quantitative methods applicable to clinical use, but as no thoroughly satisfactory test has yet been developed, we have not attempted to follow the bile acid metabolism in the present study. Miss Aldrich has recently developed in this laboratory a quantitative method that promises to be applicable to the study of the bile salts in the blood. This will be reported later.

The rôle of bile in the emulsification of ingested fat and the steatorrhea following biliary obstruction are well known. Brulé⁶⁰ and others have suggested that bile salts are essential for the absorption of fats from the intestine, and it was on this basis that his hemoconia test was devised. Normally there is an increase in ultramicroscopic blood fat (hemoconia) after a fat meal, this increase does not occur unless bile salts are present in the duodenum. Since stercobilin may appear in the stools when there is complete biliary obstruction, it would seem that this test might be of value in determining the presence or absence of bile salts in the duodenum. In our experience it has given

57 Smyth, F. S., and Whipple, G. H. Bile Salt Metabolism, I, Influence of Chloroform and Phosphorus on Bile Fistula Dogs, *J. Biol. Chem.* **59** 623-659 (April) 1924. Whipple, G. H. Origin and Significance of Constituents of Bile, *Physiological Rev.* **2** 440-457 (July) 1922.

58 Foster, M. G., and Hooper, C. W. The Metabolism of Bile Acids, I, A Quantitative Method for Analysis of Bile Acid in Dog's Bile, *J. Biol. Chem.* **38** 355-366 (June) 1919.

59 Tashiro, S. Determination of Bile Salts in the Blood (Preliminary Report), *Cincinnati J. Med.* **4** 197-201, 1923.

60 Brulé, M. Recherches sur les ictères les retentions biliaires par insuffisance hépatique, Ed. 3, Paris, Masson et Cie 1922. Castaigne, J. and Brulé, M. Foie et pancréas, in: Sergent, E., Ribadeau-Dumas, L., and Babonnet, L. Traité de pathologie médicale et de thérapeutique appliquée, Paris, A. Maloine et fils, **12**, 1920.

results comparable with the usual test for bile in the stool, although theoretically it should be somewhat more accurate

CHANGES IN THE BLOOD ACCOMPANYING DISEASE OF THE LIVER

The frequent occurrence of hemorrhages during the course of hepatic disturbances has long been known. Lee and Vincent⁶¹ more recently have emphasized the close relationship between the tendency toward postoperative hemorrhage in patients with obstructive jaundice and the delay in the coagulation time of the blood. They pointed out that the coagulation of venous blood was greatly retarded, but that the addition in vitro of a calcium chlorid solution greatly accelerated it. Walters⁶² has developed the clinical importance of this observation by showing that in such cases the intravenous administration of calcium chlorid will reduce the coagulation time of the blood to within the normal limits of from six to eight minutes. He has shown also that the routine preoperative preparation of jaundiced patients in this way greatly reduces the frequency of postoperative hemorrhages. The beneficial effect of calcium therapy has led to the suggestion that the delayed coagulation in jaundice is due to the absence of available calcium, although it is known that the total calcium content of the serum departs but little, if any, from the normal.

A decrease in fibrinogen was formerly considered a significant finding in suspected hepatic disease, but more recent observations, with improved technical methods, have necessitated a revision of opinion with regard to this point. Whipple and his collaborators⁶³ have made extensive experimental studies showing the relation of diet, inflammation and other factors to the fibrinogen content of the blood. From their work it would seem difficult to accept variations in fibrinogen as of specific diagnostic value in disease of the liver as tissue injury in general is an efficient stimulus to increased fibrin production. They conclude, however, that from all available data the liver is the only demonstrable source of fibrinogen in the body.

THE DETOXIFYING ACTIVITY OF THE LIVER

Many toxic substances gain access to the organism only to be rapidly detoxified. By oxidation or conjugation with neutral substances, these

61 Lee, R. I., and White, P. D. A Clinical Study of the Coagulation Time of Blood, *Am J M Sc* **145** 495-503, 1913; Lee, R. I., and Vincent, Beth. The Relation of Calcium to the Delayed Coagulation of Blood in Obstructive Jaundice, *Arch Int Med* **16** 59-66 (July) 1915.

62 Walters, W. Preoperative Preparation of Patients with Obstructive Jaundice, *Surg, Gynec & Obst* **33** 651-656 (Dec) 1921.

63 Foster, D. P., and Whipple, G. H. Blood Fibrin Studies, IV, Fibrin Values Influenced by Cell Injury, Inflammation, Intoxication, Liver Injury and the Eck Fistula, *Am J Physiol* **58** 407-431 (Jan) 1921.

toxic materials are converted into physiologically inert bodies that in turn are excreted through the urine or bile. The oxidation of indol to indoxyl and its ultimate excretion in the urine as indoxyl sulphuric acid, the conjugation of the toxic cholic acid with glycocholl and taurin to form glycocholic and taurocholic acids, which are excreted in the bile, and the formation and excretion of conjugated glucuronates are all evidences of this type of detoxification. The site of this detoxification is not known with certainty, but many of these changes are ascribed to the liver, and consequently there have been many attempts to secure a measure of the detoxifying power of the organism as an index to the functional activity of the liver.

Benzoic acid is detoxified in the organism and excreted as hippuric acid. The liver has been thought to be active in this synthesis, although the kidney apparently is the chief locus of hippuric acid formation. A combined clinical and experimental study of the benzoate test has recently been made by Bryan⁶⁴ in this laboratory, and will be reported shortly.

At the time Strauss introduced his fructose tolerance test, he also attempted to measure the detoxifying power of the liver by feeding a known amount of indol and measuring the excretion of indican in the urine. Foster and Kahn⁶⁵ fed thymol and measured the increase in the percentage of ethereal sulphates in the urine as an index to the degree of conjugation of the thymol.

Other phenolic substances including camphor, salicylic acid, cresol, guaiacol and menthol have all been used to provoke conjugation. The greatest measure of success with tests of this type has been reported by Roger and Chiray,⁶⁶ who gave a provocative dose of camphor and measured the excretion of camphor glucuronic acid. Conjugation was either delayed or diminished in patients with hepatic disorders.

Delprat and Whipple⁶⁷ have recently reviewed studies of liver functional tests and reported some of their own experimental studies. More recently Pelkan and Whipple⁶⁸ have investigated the appearance of conjugated phenols in the blood following the administration of p-cresol.

64 Bryan, A. W. Clinical and Experimental Studies on Sodium Benzoate, I, The Value of the Sodium Benzoate Test of Renal Function and the Effect of Injury to the Liver on Hippuric Acid Synthesis, *J Clin Invest*, to be published.

65 Foster, C. S., and Kahn, Max. A Study of the Tests of Liver Function, *J Lab & Clin Med* **2** 25-36 (Oct) 1916.

66 Roger, H., and Chiray, M. La glycuronurie normale et pathologique, ses variations dans la cirrhose et le diabete, *Bull Acad de med* **73** 446-449, 1915.

67 Delprat, G. D., and Whipple, G. H. Studies of Liver Function, Benzoate Administration and Hippuric Acid Synthesis, *J Biol Chem* **49** 229-246 (Nov) 1921.

68 Pelkan, K. F., and Whipple, G. H. Studies of Liver Function, III, Phenol Conjugation as Influenced by Liver Injury and Insufficiency, *J Biol Chem* **50** 513-526 (Feb) 1922.

They found that the conjugation of the phenols in the body was not disturbed by bleeding, by the presence of a biliary fistula, or by a lethal intoxication (distemper). On the other hand, an Eck fistula reduced both the amount and speed of phenol conjugation. Partial exclusion of the liver by ligation of the hepatic artery also reduced conjugation. Slight injury, as a result of chloroform or phosphorus poisoning, was without effect, but extensive injury produced progressively more striking results until, with fatal injury, the phenol conjugation was reduced to zero. These observations led Pelkan and Whipple to the conclusion that phenol conjugation was a function of parenchymal cells of the liver, and of no other body cells. Mann⁶⁹ finds, however, that an appreciable degree of phenol conjugation may take place even after complete hepatectomy.

Handel,⁷⁰ who studied the excretion of guaiacol sulphuric acid and of camphor glucuronate in patients with hepatic disease, did not find constant deviations from normal. Schmid,⁷¹ in a recent report on the camphor test, concludes that it cannot be used as an index to functional changes in the liver, for the locus of conjugation is not known with certainty, the degree of conjugation is not uniform, and it is not possible quantitatively to determine the excretion of glucuronates. In view of the manifest objections we can conclude only that more knowledge concerning the exact mechanism and locus of detoxification in the body is necessary before a study of the conjugating power of the organism can be accepted clinically as an index to the functional activity of the liver.

THE WIDAL HEMOCLASTIC CRISIS

Widal⁷² recently has suggested that an important function of the liver is the detoxification of protein-split products, such as proteoses and peptones, formed in the intestinal canal during digestion. Failure of this proteopexic activity of the liver permits the passage of such products into the general circulation where an anaphylactic reaction is produced. This last is evidenced by changes in the blood pressure, but especially by a transitory leukopenia, the hemoclastic crisis.⁷² The usual practice is to make a leukocyte count after a fast of at least five hours.

69 Mann, F. C. Personal communication to the authors.

70 Handel, M. Klinisch-experimentelle Studien über die entgiftende Funktion der Leber, I, Ueber Schwefelsäure- und Glukuronsäurepaarung bei Leberkranken, *Ztschr. f. d. ges. exper. Med.* **42** 172-193, 1924.

71 Schmid, F. L'épreuve de la fonction hépatique par la glucuronurie provoquée, *Ann. de med.* **13** 328-358 (April) 1923.

72 Widal, F., Abrami, P., and Iancovescu, N. L'épreuve de l'hémoclasie digestive dans l'étude de l'insuffisance hépatique, *Presse med.* **2** 893-898, 1920, Feinblatt, H. M. Alimentary Leukocytosis in Various Pathologic Conditions. A Further Study in Reference to the Crise hémoclasique of Widal, *Arch. Int. Med.* **33** 210-216 (Feb.) 1924.

The subject is then given 200 c c of milk, and the leukocyte count repeated at half-hour intervals. Normally the usual postalbuminary leukocytosis follows, but leukopenia is observed in a variable proportion of subjects in both health and disease. The significance of the test is uncertain and many observers are agreed as to the great variability in the response. We have been unable satisfactorily to correlate the test with other clinical and laboratory evidences of hepatic disturbance, and question its value in the diagnosis of disease of the liver.

PHENOLTETRACHLOROPHTHALEIN

Phenoltetrachlorophthalein was first prepared by Orndorff and Black⁷³ in 1909. Pharmacologic studies by Abel and Rowntree⁷⁴ showed that, when given by mouth to healthy animals, the drug was not absorbed from the intestinal tract, but when given parenterally in moderate doses either intravenously or subcutaneously, dissolved in oil, the dye was excreted solely in the bile. The specificity of this path of excretion suggested the use of phenoltetrachlorophthalein as a test for hepatic function.

This test, as originally described by Rowntree, Hurwitz and Bloomfield, was carried out by the intravenous injection of a solution containing 400 mg of the dye. Active purgation was instituted and the total feces were collected for a period of forty-eight hours. The total amount of dye in the specimen of stool was then determined. In a series of normal patients, from 30 to 50 per cent of the dye injected was recovered in the stools. From patients with disease of the liver the proportion of dye recovered was significantly decreased.

Whipple, Peightal and Clark⁷⁵ studied the output of phenoltetrachlorophthalein in experimental animals, and found that the degree of hepatic damage produced in dogs by chloroform or phosphorus poisoning or cautery of the liver was paralleled by the drop in the excretion of the dye.

Subsequent opinions regarding the original test have varied greatly. Rowntree, Marshall and Chesney,¹ in 1915, reported another series of cases, extending and amplifying the original report. Krumbhaar⁷⁶ likewise considered the test of definite value. On the other hand,

73 Orndorff, W. R., and Black, J. A. Phenoltetrachlorophthalein and Some of Its Derivatives, *Am Chem J* **41** 349-393, 1909, *Chem Abst* **3** 1875-1876, 1909.

74 Abel, J. J., and Rowntree, L. G. On the Pharmacological Action of Some Phthaleins and Their Derivatives, with Especial Reference to Their Behavior as Purgatives, *I, J Pharm & Exper Therap* **1** 231-264, 1909.

75 Whipple, G. H., Peightal, T. C., and Clark, A. H. Tests for Hepatic Function and Disease Under Experimental Conditions. Phenoltetrachlorophthalein, *Bull Johns Hopkins Hosp* **24** 343-357, 1913.

76 Krumbhaar, E. B. The Present Status of Functional Liver Tests, *New York M J* **100** 719-721, 1914.

Sisson,⁷⁷ McLester and Frazier⁷⁸ and Kahn and Johnston,⁷⁹ who reported smaller series of cases, were doubtful of the value of the test

The original technic of Rowntree, Hurwitz and Bloomfield² was time consuming and the collection and analysis of the specimens of stools unpleasant and difficult. These objections led McNeil,⁸⁰ in 1915, to abandon entirely the collection of stools. He substituted for it the passage of a duodenal tube, and the determination of both the time interval elapsing between the injection of the dye and its appearance in the duodenal drip, and the amount of dye recovered during a two-hour period of drainage. McNeil, and Piersol and Bockus⁸¹ report that the time of the appearance and the total output of the dye are related. Deakin and Graham⁸² maintained that the uncertainty regarding the quantitative recovery of bile through the duodenal tube militated against any study of the total output of phenoltetrachlorophthalein, and this view is now widely accepted. The normal time of appearance of the dye in the duodenal drip varies between fifteen and twenty-five minutes. Numerous observers, including Kahn,⁸³ Aaron, Beck and Schneider,⁸⁴ Williams,⁸⁵ Piersol and Bockus,⁸¹ Higgins⁸⁶ and Friedenwald and Gantt⁸⁷ are agreed that pathologic cases usually show sufficient delay in the time of appearance to make the test of distinct clinical value

77 Sisson, W. R. A Clinical Study of Two Hepatic Functional Tests (Galactose and Phenoltetrachlorophthalein), *Arch Int Med* **14** 804-826 (Dec) 1914

78 McLester, J. S. and Frazier, Blanche. Phenoltetrachlorophthalein Test of Liver Function in a Series of Unselected Cases, *J. A. M. A.* **65** 383-387 (July 31) 1915

79 Kahn, M., and Johnston, J. R. The Phenoltetrachlorophthalein Test of Liver Function, *New York M. J.* **102** 848-850, 1915

80 McNeil, H. L. The Quantitative Estimation of Phenoltetrachlorophthalein Excreted in the Fresh Bile in Disease of the Liver, *J. Lab. & Clin. Med.* **1** 822-825, 1915

81 Piersol, G. M., and Bockus, H. L. The Value of Phenoltetrachlorophthalein in Estimating Liver Function, *Tr. A. Am. Phys.* **37** 433-451, 1922, *Arch Int Med* **31** 623-636 (May) 1923, Comparative Studies in Liver Function by Some of the Later Methods, *J. A. M. A.* **83** 1043-1049 (Oct 4) 1924

82 Deakin, V. R., and Graham, E. A. Functional Liver Tests, an Experimental Study, *Surg., Gynec. & Obst.* **36** 348-354 (March) 1923

83 Kahn, Max. Phenoltetrachlorophthalein Estimation in the Duodenal Contents, *J. A. M. A.* **77** 41 (July 2) 1921

84 Aaron, A. H., Beck, E. C., and Schneider, H. C. The Phenoltetrachlorophthalein Test for Liver Function, *J. A. M. A.* **77** 1631-1634 (Nov 19) 1921

85 Williams, P. F. The Phenoltetrachlorophthalein Test for Liver Function in Pregnancy. *Am. J. Obst. & Gynec.* **4** 26-30 (July) 1922

86 Higgins, C. C. Observations upon the Phenoltetrachlorophthalein Test for Liver Function, *Ann. Clin. Med.* **2** 30-18 (July) 1923

87 Friedenwald, J., and Gantt, W. H. Some Observations on the Phenoltetrachlorophthalein Test as a Means of Determining Liver Function, *Am. J. M. Sc.* **166** 519-526 (Oct) 1923

Hoxie,⁸⁸ on the other hand, does not consider the time of appearance to be sufficiently uniform for routine clinical use. The arguments for and against the study of the appearance of phenoltetrachlorophthalein apply with equal force to the use of other dyes, such as methylene blue or rose bengal, in chromocholoscropy.

The difficulties and impracticability of studying the excretion of phenoltetrachlorophthalein in the stools or duodenal contents were later emphasized by Rosenthal.⁸⁹ After the intravenous injection of the dye, he found it to be removed from the blood stream very rapidly and uniformly. In normal persons only from 2 to 7 per cent of the dye was found in the blood serum fifteen minutes after the injection, and less than 3 per cent at the end of an hour. Experimental studies by Rosenthal showed that after phosphorus or chloroform poisoning or partial removal of the liver there was a marked retention of the dye in the blood stream. Lamson⁹⁰ has also used this method with success in his study of the toxic effects of carbontetrachlorid.

As the result of his experimental studies, Rosenthal suggested the rate of removal of phenoltetrachlorophthalein from the blood stream as the most satisfactory clinical test of hepatic disturbance. Rosenfield and Schneiders,⁹¹ and Greenbaum and Brown⁹² have reported small series of cases indicating the value of this test in the toxemias of pregnancy and in syphilitic affections of the liver. In the present study, the rate of removal of this drug from the blood stream was determined according to the principles outlined by Rosenthal. Because of minor variations in the technic used we shall describe our method in detail.

88 Hoxie, G. H. Phenoltetrachlorophthalein Liver Function Test, *J. A. M. A.* **82** 361-362 (Feb. 2) 1924.

89 Rosenthal, S. M. An Improved Method for Using Phenoltetrachlorophthalein as a Liver Function Test, *J. Pharm. & Exper. Therap.* **19** 385-391 (June) 1922, A New Method of Testing Liver Function with Phenoltetrachlorophthalein, II, *Bull. Johns Hopkins Hosp.* **33** 432-437 (Dec.) 1922, A New Method of Testing Liver Function with Phenoltetrachlorophthalein, III, Clinical Report, *J. A. M. A.* **79** 2151-2154 (Dec. 23) 1922, A New Method of Testing Liver Function with Phenoltetrachlorophthalein, IV, The Relation of Impaired Function to the Amount of Normal Liver Tissue, *J. Pharm. & Exper. Therap.* **23** 385-393 (June) 1924, *Proc. Soc. Exper. Biol. & Med.* **21** 73-75, 1923-1924, The Phenoltetrachlorophthalein Test for Hepatic Function, Recent Studies with the Author's Method, *J. A. M. A.* **83** 1049-1053 (Oct. 4) 1924.

90 Lamson, P. D., Gardner, G. H., Gustafson, R. K., Maire, E. D., McLean, A. J., and Wells, H. S. The Pharmacology and Toxicology of Carbon Tetrachlorid, *J. Pharm. & Exper. Therap.* **22** 215-288 (Nov.) 1923, The Toxicity of Carbon Tetrachlorid in Relation to Liver Function as Tested by Phenoltetrachlorophthalein, *J. Pharm. & Exper. Therap.* **21** 237-246 (May) 1923.

91 Rosenfield, H. H., and Schneiders, E. F. Improved Phenoltetrachlorophthalein Test for Liver Function in Pregnancy and Its Toxemias, *J. A. M. A.* **80** 743-747 (March 17) 1923.

92 Greenbaum, S. S., and Brown, H. The Phenoltetrachlorophthalein Liver Test, in Cases of Acute and Chronic Syphilis Under Treatment and in Various Skin Diseases, *J. A. M. A.* **82** 88-91 (Jan. 12) 1924.

Method of Determining Rate of Removal of Phenoltetrachlorophthalein from the Blood Stream—The phenoltetrachlorophthalein is injected intravenously in a dosage of 5 mg for each kilogram of body weight. This corresponds to 1 cc of the solution prepared according to the original directions of Rowntree, Hurwitz and Bloomfield for each 10 kg of body weight.⁹³ If a large superficial vessel in which there is a free flow of blood, such as the median cubital vein, is chosen and the undiluted dye solution is injected slowly enough (from 1 to 2 cc each minute) to permit of considerable dilution by the blood stream during the injection, no reaction follows. If the injection is made into one of the other superficial veins, such as the cephalic, in which the blood flow is not so great, or if the injection is made too rapidly, there may be a sensation of a transient burning along the course of the vein. Occasionally slight induration or local thrombosis at the site of injection occurs, especially if the dye solution is allowed to leak back from the vein into the subcutaneous tissues.

The majority of observers who have injected phenoltetrachlorophthalein in amounts similar to those used in this study have diluted the dye with distilled water or salt solution. Such dilution requires the administration of the dye by gravity or the use of some special three-way stopcock to permit the washing out of the system. The simplicity of the apparatus and the resultant ease of sterilization and administration, we believe, commends the direct method of injection. On account of the irritant action of the dye solution, the greatest care and exactitude are required in its administration.

Following the injection of the phenoltetrachlorophthalein solution, blood samples are taken at fifteen minutes, one hour and two hours. These samples are preferably taken from the other arm in order to avoid the possibility of contamination from the retention of dye at the site of injection, although this danger is negligible in practice. We have noticed no difference in the readings of serum and plasma samples provided they are unhemolyzed.

The amount of dye in the serum is most easily determined by dividing the sample between two tubes of equal caliber. A trace of alkali suffices to develop the maximal color of the dye in one tube, while the other serves as a control. Comparison is then made against standard tubes of dye, using the Walpole⁹⁴ method of compensating for the serum color. Hemolyzed bloods are precipitated by acetone, as suggested by Bloom.

⁹³ Ampules containing a stable solution of phenoltetrachlorophthalein of this concentration are now prepared by Hynson, Westcott & Dunning.

⁹⁴ Walpole, G. S. Chart Presentation on Recent Work on Indicators, *Biochem J* 5 207-214, 1911.

and Rosenau⁹⁵ The extraction of dye by acetone is not entirely complete, but the method is satisfactory for ordinary purposes We have adhered to Rosenthal's original method⁹⁶ of notation in reading the dye in percentage of an assumed initial concentration Variations in the plasma volume introduce minor inaccuracies in this system of notation, but this error is too slight to be significant and may be disregarded

The comparison of different patients or of a series of tests on the same patient necessitates a uniform notation of the degree of dye retention indicated by each test Lamson⁹⁰ has introduced the conception of a "toxicity index" based on the total area subtended by the curve showing the concentration of dye in the plasma The accurate determination of this curve of concentration requires repeated venipuncture up to the time of disappearance of the dye from the plasma, and the use of a planimeter for the measurement of the area subtended by the resultant curve Lamson has pointed out the difficulties in the use of this toxicity index, and admits that at best the values are approximate only

We have preferred to refer to positive readings as indicating slight, moderate, marked and maximal degrees of retention Both the concentration of the dye at the initial reading and the rate of removal from the blood stream are considered in this determination of degree of retention When a comparison of several readings has been made, we have usually taken the one-hour readings This is the most valuable single sample, but our knowledge of the test is not as yet sufficient to warrant the entire dependence on this one sample, as suggested by Bogen⁹⁶

Urinary Excretion of Phenoltetrachlorophthalein—Rowntree, Hurwitz and Bloomfield mentioned the urinary excretion of phenoltetrachlorophthalein in their original papers They did not find the dye in the urine of any normal subject, and believed that its appearance in the urine was of distinct clinical significance Subsequent investigators either have not studied the urinary output, or else have not considered it significant

As a matter of routine we have collected the urine over a period of two hours following the injection of phenoltetrachlorophthalein Traces of the dye may appear in the urine of normal subjects, but only in exceptional instances does the total output exceed 0.5 mg The urinary output apparently is dependent on the concentration of the dye in the blood, and amounts of 1 mg or more are indicative of definite retention

95 Bloom, W, and Rosenau, W H A Simple Method for the Determination of Phenoltetrachlorophthalein in Blood Serum, *J A M A* **82** 547 (Feb 16) 1924

96 This method of determining the content of dye has been developed independently by Bogen (A Clinical Test for Liver Function, *J Lab & Clin Med* **8** 619-621 [June] 1923), Broun, and Rosenthal The standard tubes of phenoltetrachlorophthalein fade readily when exposed to light and may well be replaced by the inorganic standard solution developed by Hynson, Westcott & Dunning

of dye It must be remembered that the renal excretion is dependent partly on the functional activity of the kidneys, and patients with combined hepatic and renal disease may not show a urinary excretion of phenoltetrachlorophthalein commensurate with the degree of retention in the blood In doubtful cases, however, the urinary findings may be of distinct clinical value

BROMSULPHALEIN

Recently, White ⁹⁷ has prepared a disulphonic derivative of phenoltetrabromphthalein The disodium salt of this derivative (bromsulphalein ⁹⁷) is readily soluble in water, forming a colorless, neutral, nonirritating solution The addition of an excess of alkali suffices to produce the characteristic reddish violet of the original dye The physiologic properties of this new derivative apparently are very closely allied to those of the phenoltetrachlorophthalein, but because of the neutral and therefore nonirritating character of the solution, the disulphonic acid derivative may prove of superior usefulness for routine use The latter dye leaves the blood stream somewhat less rapidly than the original, and accordingly is a more sensitive indicator of small degrees of change

COMMENT

We have here tried to give an outline of the reported tests for those physiologic changes in the liver which gave sufficient evidence of clinical usefulness to warrant further study In the subsequent papers of this series, we shall report the results of a comparative study of certain of these tests in various clinical and experimental conditions

⁹⁷ Rosenthal, S M., and White, E C Studies in Hepatic Function, VI A, The Pharmacological Behavior of Certain Phthalein Dyes, B, The Value of Selected Phthalein Compounds in the Estimation of Hepatic Function, *J Pharm & Exper Therap* **24** 265-288 (Nov) 1924, Clinical Application of the Bromsulphalein Test for Hepatic Function, *J A M A* **84** 1112-1114 (April 11) 1925

DISEASES OF THE LIVER

II A COMPARATIVE STUDY OF CERTAIN TESTS FOR HEPATIC FUNCTION IN EXPERIMENTAL OBSTRUCTIVE JAUNDICE *

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Study I of this series was devoted to a consideration of the physiologic basis, technic and interpretation of a number of clinical tests for functional disturbances of the liver¹ The present study of tests for hepatic function in experimental obstructive jaundice was undertaken with the view of (1) delimiting the relative value and significance of the various tests under controlled conditions, (2) establishing an experimental background for the better interpretation of the functional deviations clinically observed in patients with obstructive jaundice, and (3) correlating, so far as possible, the physiologic changes indicated by the various tests with the pathologic changes observed at necropsy

Two groups of six dogs each were studied those which had had simple ligation of the common duct, and those which had had cholecystectomy besides ligation of the common duct In all cases complete studies with regard to the tests to be employed were made before the operation, and at intervals of from one to three days during the period immediately following the production of the obstruction With the development of marked jaundice there were definite changes in the functional tests Subsequently, the changes were less rapid, and tests were made at weekly intervals A second operation was performed in a few instances to relieve the obstruction, and the course of the recovery was studied

The animals were kept on a constant diet of meat, milk and dog biscuit Following ligation of the common duct, the dogs of the first group manifested but little postoperative reaction and were in good condition for the first three to five days, when icterus became prominent, thereafter they developed lassitude, anorexia, weakness and progressive emaciation The animals of the second group, in which the gallbladder was removed at the time the common duct was ligated, developed a profound intoxication and icterus within twenty-four hours after operation

* Read before the Society of Clinical Surgery, Rochester, Minn., June 7, 1924

¹ From the Division of Medicine, Mayo Clinic and Mayo Foundation

1 Greene, C H., Snell, A M., and Walters, Waltman Clinical and Experimental Studies in Diseases of the Liver, I, A Survey of Tests for Hepatic Function, Arch Int Med **36** 248 (Aug) 1925

These dogs usually improved somewhat after a few days, and thereafter the clinical condition of the animals of the two series was similar.

Control experiments were made to determine the specific effects of the anemia, acidosis, anorexia and loss of weight incidental to the experimental procedures. A few animals were fed daily by stomach tube a mixture of corn syrup and milk, to prevent the loss of weight following operation. These animals remained in a somewhat better condition than those allowed to eat at random, but the changes in the responses to the different tests were identical in the two series. In further control experiments on normal animals, the effects were studied of fasting that caused a loss of 40 per cent of the initial body weight, of acidosis produced by a ketogenic diet and the administration of ethyl aceto-acetate and of anemia produced by either a single large bleeding or several small bleedings. In general the latter procedure had but little effect on the results of the different tests.

DATA IN EXPERIMENTAL JAUNDICE

The functional disturbances observed following the production of obstruction were strikingly uniform in both series of experiments. Five animals in which complete studies were made were included in each series. Slight individual variations occurred, but the results, as exemplified by these animals, were quite constant for each experimental series.

PROTOCOLS

EXPERIMENT 1—Dog H26 was an adult male, black and white mongrel in good condition, weighing 13.4 kg. March 31, a complete series of functional tests was performed. April 1, the phenoltetrachlorophthalein test was repeated as an additional control. April 3, under ether anesthesia and using aseptic technic, the common duct was doubly ligated and sectioned between clamps. The gallbladder was not disturbed. April 4, the animal was in good condition and without evidence of postoperative reaction. The functional studies were repeated (Fig. 1). April 7, slight icterus was apparent in the skin and conjunctiva. There was then a gradual increase in the degree of apparent icterus associated with progressive anorexia and loss of weight. The findings in the different tests are shown in Table 1. May 6, an attempt was made to relieve the obstruction. At operation the cut end of the obstructed common duct was readily found, as it was dilated to many times the normal diameter. An anastomosis was made between the dilated duct and a loop of the ileum. The operation was difficult and there was some unavoidable soiling of the operative field. The animal did not do well and died twenty-eight hours later. Functional studies were made immediately before death.

At necropsy immediately after death, the mucous membranes were jaundiced in appearance and the tissues bile stained, especially the intimal lining of the heart and aorta. Only small amounts of dirty yellow omental fat were observed. There was no free fluid in the peritoneal cavity. The anastomosis was intact and the intestine contained bile. The extrahepatic bile ducts were greatly dilated, and the thin-walled, distended gallbladder contained thick viscid green bile. The liver was shrunken, congested and mottled in appearance. The intrahepatic bile ducts were moderately dilated. The mesenteric lymph

nodes were enlarged, and there was an acute pancreatitis with multiple areas of fat necrosis. The spinal fluid and aqueous humor were colorless, and qualitative tests for bile pigments were negative.

Microscopic examination of the liver (Fig 2) disclosed a slight increase in connective tissue around the bile ducts with beginning round cell infiltration. In the central portion of the lobule there was a slight staining from bile, and a few bile thrombi were present in the canaliculi. The parenchymal cells were swollen, the sinuses compressed, and there was no increase in the endothelial cells.

EXPERIMENT 2—Dog H25 was an adult, male mongrel bulldog in good condition, weighing 124 kg. March 31, a complete series of functional tests was performed. April 1, the phenoltetrachlorophthalein test was repeated as an additional control. April 3, under ether anesthesia and using aseptic technique, a cholecystectomy was performed and the common duct doubly ligated and sectioned between clamps. The following day the dog was definitely jaundiced, extremely ill, and scarcely able to stand. There was bile in the urine.

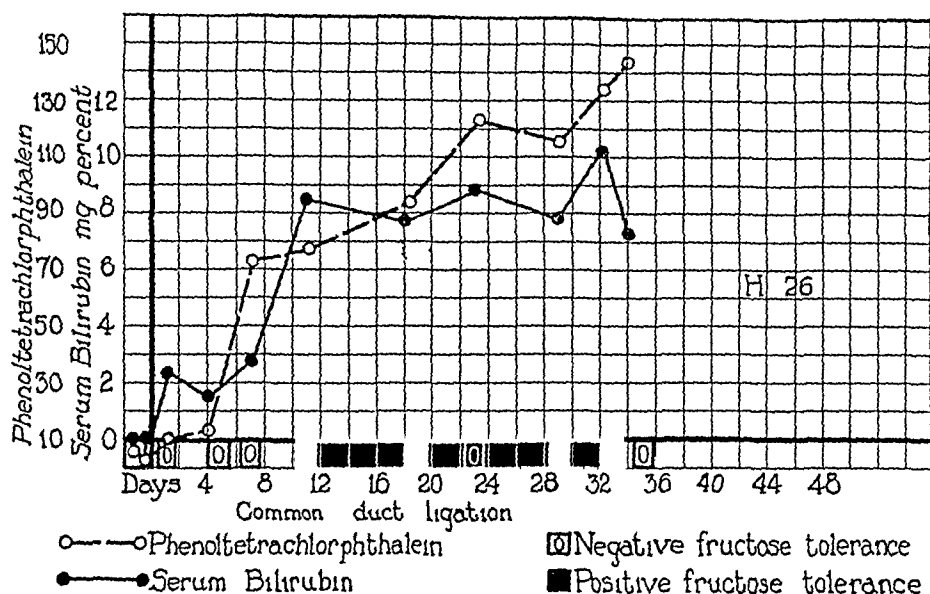


Fig 1 (Dog H26)—Changes in serum bilirubin, phenoltetrachlorophthalein and fructose tolerance tests following ligation of the common bile duct.

Functional studies were made (Fig 3). April 5, the animal was somewhat better. The stools were very light in color. April 8, the animal was much improved and was eating well, but thereafter emaciation constantly increased, and there were icterus and anorexia (Table 2). The dog was found dead in the cage fifty-five days after the operation.

At necropsy the mucous membranes, sclerae, subcutaneous fat and linings of the heart and blood vessels were deeply tinged with bile. There was no free fluid in the peritoneal cavity and no evidence of peritonitis. The liver was shrunken and dark, weighing only 212 gm, and showed marked resistance on section. Both the extrahepatic and the intrahepatic bile passages were markedly dilated and filled with dark, viscid bile. The kidneys were swollen, and the cortex was bile stained.

Microscopic examination of the liver (Fig 4) disclosed that the normal architecture was not disturbed, although there was considerable fibrous tissue thickening around the branches of the hepatic ducts. The bile ducts were thickened and there was much surrounding round cell infiltration. Staining from bile was marked in the central portion of the lobule, the canaliculi being filled with pigment masses. The hepatic cells were small and atrophic. The

TABLE 1—Changes Observed Following

Date	Time After Operation, Days	Weight, Kg	Urine				Hemoglobin, per Cent	Blood Nitrogen Partition, Mg, per Cent				Bile Pigments (Serum)		
			Icterus*	Albumin*	Bile*	Casts*	Phenolsulphone phthalein, per Cent	Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen	Bile Index	Van den Bergh Direct Reaction
3/31/24		13.4	0		0		60	35	41	Low†	11	80	0	0
4/ 1/24		13.4	0											
4/ 4/24	1	12.7	Trace	2	0	0		107	23	Low†	13			0
4/ 7/24	4	11.7	1	2	2	0		108	24	Low†	13	68		+
4/10/24	7	11.3	2	0	2	0	40	108	21	Low†	14		34	+
4/14/24	11	10.5	2		2			25	25	Low†	0.9	83	78	+
4/21/24	18	10.3	2	2	2	0		31	40	Low†	1.7	80	57	+
4/26/24	23	10.3	2				50	30	17	Low†	1.2	82	63	+
5/ 2/24	29	10.1	2+	2+	2	0	66	30	21	Low†	1.3	81	95	+
5/ 5/24	32	10.0	2+	1	2	0		31	21	Low†	1.5	95	91	+
5/ 7/24	34	9.1	2	2	2	0		70	116	Low†	1.4	98	80	+

* Graded on a basis of from 0 to 4

† Too low to read

TABLE 2—Changes Observed Following Ligation of

Date	Time After Operation, Days	Weight, Kg	Urine				Hemoglobin, per Cent	Blood Nitrogen Partition, Mg, per Cent				Bile Pigments (Serum)		
			Icterus*	Albumin*	Bile*	Casts*	Phenolsulphone phthalein, per Cent	Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen	Bile Index	Van den Bergh Direct Reaction
3/31/24		12.4	0	0	0		50	33	24	Low†	13	86	0	0
4/ 1/24														
4/ 4/24	1	11.4	Trace	Trace	1	0		31	14	Low†	0.9	68	51	+
4/ 5/24	2	11.0	2	2	2	0		29	19	1.5	1.2	73	72	+
4/ 8/24	5	10.5	2	1	2	0		27	16	Low†	1.3	76	77	+
4/11/24	8	10.0	2	0	2	0	75	27	16	Low†	0.5	67	57	+
4/16/24	13	10.2	2	Trace	2			37	21	Low†	1.2	98	44	+
4/22/24	19	10.0	2	Trace	2	0	50	25	13	Low†	1.3	56	40	+
4/28/24	25	9.2	2	Trace	2			29		Low†	1.2	65	63	+
5/ 5/24	32	9.3	2	1	2	0		25	17	Low†	1.2	70	50	+
5/12/24	39	9.1	2	1+	2	0		30	24	Low†	1.2	65	75	+
5/20/24	47	8.2	2	1+	2	1	80	33	31	Low†	1.4	76	100	+
5/26/24	53	7.4	2	1+	2	0		40	37	Low†	1.2	95	90	+

* Graded on a basis of from 0 to 4

† Too low to read

TABLE 3—Changes Observed Following Ligation of the

Date	Time After Opera- tion, Days	Weight, Kg	Urine				Hemoglobin, per Cent	Blood Nitrogen Partition, Mg, per Cent					Fragility Test, Percentage Sodium Chlorid	
			Albumin*	Bile*	Casts*	Phenolsulphone phthalein, per Cent		Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen	Initial Hemolysis	Complete Hemolysis
4/17/24		12.1	1	0	0	60	125	33	28	Low†	3.7	7.5	0.48	0.34
5/ 2/24	1	10.9	1	1	1		122	27	23	Low†	1.8	6.2	0.42	0.28
5/ 6/24	5	11.0	1	1	0			28	30	Low†	1.4		0.42	0.28
5/ 9/24	8	11.0	Trace	+	0	54		39	39	Low†	1.3	6.4	0.42	0.26
5/15/24	14	10.0	Trace	+	0		80	28	41	Low†	1.4	6.7	0.40	0.28
5/16/24	15					60								
5/18/24	17	10.7	Trace	+	0			27	42	Low†	1.3	7.2		
5/20/24	19						65							
5/21/24	20	10.1	1	+	0			25	19	2.4	1.2		0.40	0.26
5/22/24	21													
5/23/24	22	10.5	Trace	—	0	60	65	22	21	Low†	0.9	7.2	0.44	0.24
5/24/24	23													
5/26/24	25	9.5	0	—	0		60			Low†	0.9		0.42	0.28
5/28/24	27													

* Graded on a basis of from 0 to 4

† Too low to read

Ligation of the Common Bile Duct (Dog H26)

Calcium, Mg, per Cent	Coagulation Factors				Fructose Tolerance, Blood Sugar, Mg, per Cent					Phenoltetrachlorophthalein						Dye in Urine Mg
	Fibrin, Mg, per Cent		Coagulation Time, Min	Calcium Time, Minutes	Initial	15 Minutes	1 Hour	2 Hours	Change	Dye in Serum, per Cent						
	Whole Blood	Plasma								5 Minutes	10 Minutes	15 Minutes	1 Hour	2 Hours		
10.6	302	620	4.5	3.0	117	126	145	116	28	4	1	1			Trace	
	793	1,210	4.5	3.0	124	130	130	142	18	5	1	0	0	0		
9.8	512	887	8.5	5.5	237	167	123		114	7	2	2	0	0	0.2	
10.7			9.5	7.5	90	118	119	100		29	2	2	2	0		
	559	908	10.0	5.0	100	107	121	136	36	20	17	14	11	10		
11.0	930	1,503	12.0	5.0	97	138	118	110	41	32	16	13	13	13	4.5	
	686	1,160	8.0	8.5	100	130	118	115	30	35	24	23	15	11	3.7	
10.8	628	975	9.0	7.5	98	120	135	124	37	30	30	30	25	11	1.1	
10.9	483	780	12.0	6.0	100	149	149	115	49	44	30	24	24	23	0.9	
10.4	729	1,175	15.0	16.0	80	88	82	79	8	40	25	23	25	20		

the Common Bile Duct and Cholecystectomy (Dog H25)

Calcium, Mg, per Cent	Coagulation Factors		Fructose Tolerance, Blood Sugar, Mg, per Cent					Phenoltetrachlorophthalein						Dye in Urine Mg
	Fibrin, Mg, per Cent		Initial	15 Minutes	1 Hour	2 Hours	Change	Dye in Serum, per Cent						
	Whole Blood	Plasma						5 Minutes	10 Minutes	15 Minutes	1 Hour	2 Hours		
10.7			100	110	135	110	35	4	2	1	0	0	0	0.5
	507	1,015						6	5	0	0	0	0	
	753	1,355	123	130	147	120	24	10	12	5	6	6	6	0.2
			100	107	133	105	33	15	12	12	12	13	13	0.1
			80	85	100	107	27	12	10	12	8	8	8	4.2
10.1	748	1,265	103	146	144	164	43	16	13	10	6	5	5	1.1
	885	1,200	106	135	148	146	42	14	14	13	8	4	4	1.3
10.5	800	1,115	95	118	133	124	38	18	12	12	12	10	10	1.7
10.8	822	1,300	92	120	130	10	38	26	13	13	12	10	10	1.6
11.0	569	860	95	97	148	117	53	27	20	0	12	12	12	
10.4	1,441	2,515	88	111	140	145	57	16	16	10	10	9	9	1.5
10.4	669	1,115	79	87	190	180	111	22	25	25	15	8	8	2.8
10.2			83	87	125	137	54	22	20	11	10	10	10	4.9

Common Bile Duct and Cholecystectomy (Dog H32)

Bile Pigments (Serum)			Coagulation Factors				Phenoltetrachlorophthalein						Remarks
Bile Index	Serum Bilirubin, Mg, per Cent	Van den Bergh Direct Reaction	Fibrin, Mg, per Cent			Hemoconia	Dye in Serum, per Cent					Dye in Urine, Mg	
			Calcium, Mg, per Cent	Whole Blood	Serum		5 Minutes	10 Minutes	15 Minutes	1 Hour	2 Hours		
7	0.0	0	10.7	578	1,100	++	4	3	2	Trace	0	Trace	Dog lively, no icterus
37	3.6	+	10.8	530	1,020	++	13	13	11	10	14	35	Dog quite sick
58	5.8	+	10.3	547	915	0	15	10	10	10	8	17	Icterus 1
45	4.8	+	10.9	541	875	0	15	7	6	6	7	18	Sick, tube feeding, icterus 1 to 2
85	12.5	+	10.3	518	745	0	8	7	7	7	6	12	
47	13.4	+	9.9	581	850	0	13	10	6	8	6	21	Gaining slightly
	7.8	+											Rubber T tube into common duct
20	2.4	+	9.8	450	585	+	6	5	3	1	2	0.1	Bile in stool
17	1.8	+											
11	1.7	+	9.1	406	690	++	8	5	5	4	2	0.5	
	1.5	+											Tube obstructed
	2.9	+				+	15	10	8	7	3	1.9	No bile in stool
	3.6					0							Death

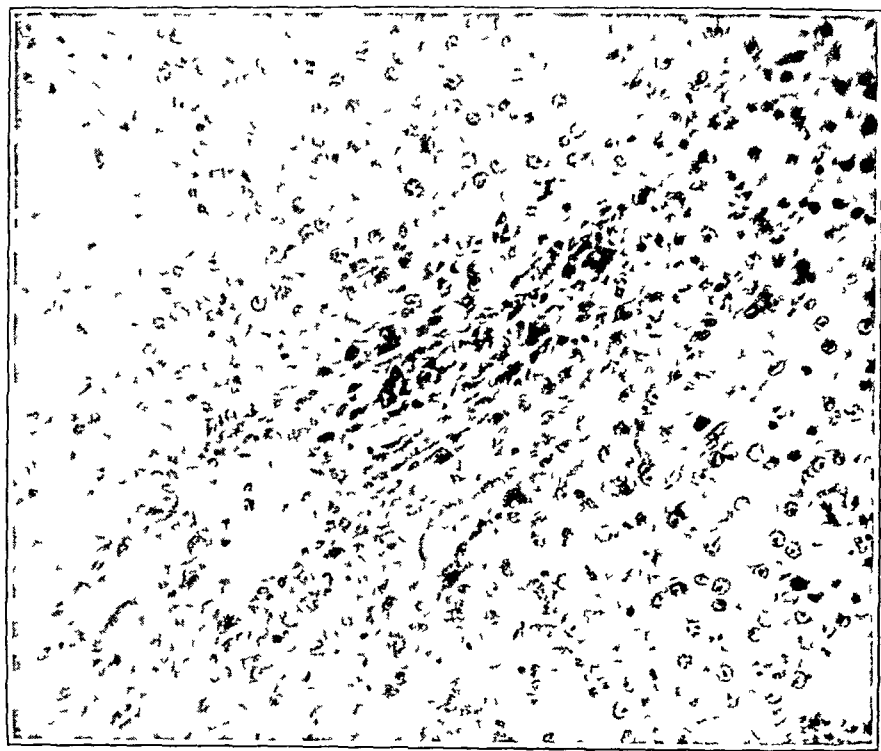


Fig 2 (Dog H26) —Section of liver showing changes thirty-four days after ligation of common bile duct

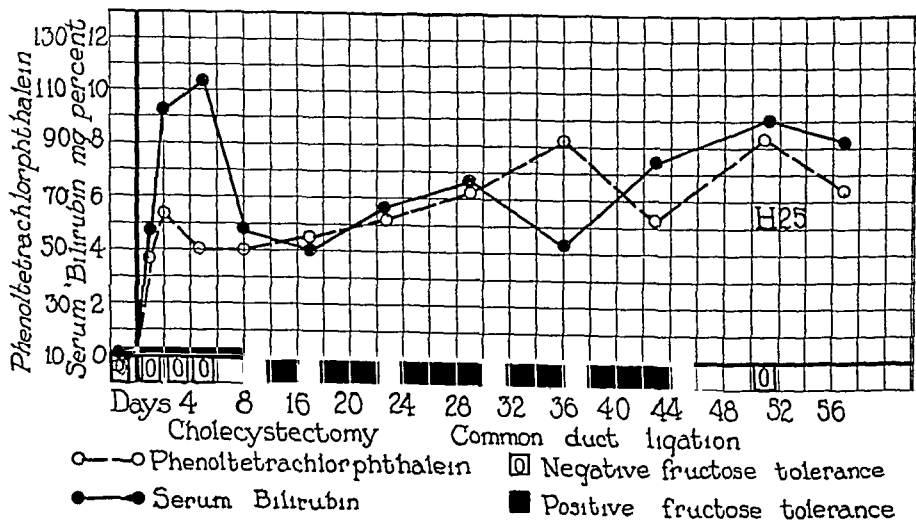


Fig 3 (Dog H25) —Changes in serum bilirubin phenoltetrachlorophthalein and fructose tolerance tests following ligation of common bile duct and removal of gallbladder

sinusoids were dilated and there was a slight increase in the endothelial tissues. The changes were much more marked than in the preceding animal.

EXPERIMENT 3—Dog H32 was an adult female, brown and white hound in good condition, weighing 121 kg. April 17, a complete series of functional tests was made (Fig 5). May 1, under ether anesthesia and aseptic technique, cholecystectomy was performed and the common duct doubly ligated and sectioned between clamps. May 2, the animal was profoundly toxic, and the sclerae and mucous membranes were definitely icteric. May 6, the animal was somewhat better, but the icterus was unchanged. Thereafter there was progressive anorexia and loss of weight (Table 3). After May 15, the animal was fed daily a mixture of milk and corn syrup which checked the loss of weight but had no other apparent effect. May 20, a second operation was performed and an anastomosis made between the two portions of the common duct by means of a T-tube. At this time the extrahepatic bile ducts were greatly dilated and

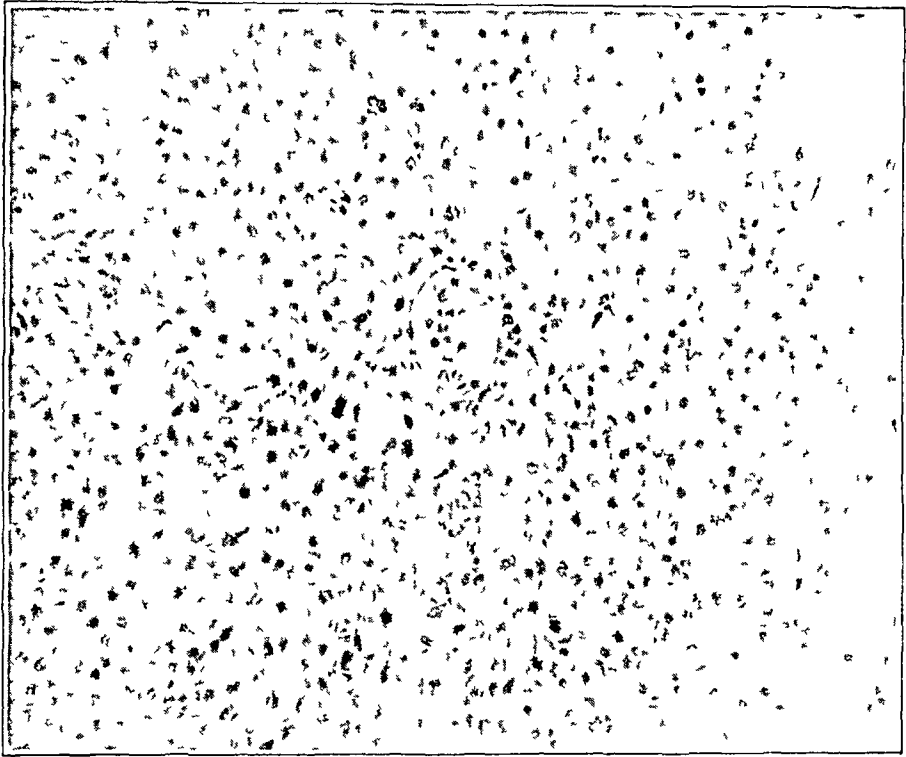


Fig 4 (Dog H25) —Section of liver showing changes fifty-three days after ligation of common bile duct and removal of gallbladder.

thin-walled, and the operation was difficult. Following the operation there was a free drainage of bile from the T-tube, and the stools became darker in color, although they did not return to normal. May 26, six days after operation, drainage from the T-tube ceased and the dog's condition was obviously worse. May 27, tests of the stool for bile were negative. The animal became progressively worse and was found dead in its cage, May 28.

At necropsy the usual icteric staining was noted. The liver was deeply stained with bile and shrunken, and tough on section. The intrahepatic bile ducts were markedly dilated. The T-tube was kinked and the proximal end completely obstructed by calcareous material.

This experiment was of particular interest in that there was a definite decrease in the serum bilirubin and the degree of dye retention following operative relief of the obstruction. The drainage tube later became blocked, with consequent development of the changes characteristic of obstruction. These changes were similar to those seen clinically under similar circumstances.

DISCUSSION OF TESTS

Fructose Tolerance—The changes in the fructose tolerance were studied by the technic of Bodansky² The dogs were given 3 gm of sugar for each kilogram of body weight, and the blood sugar determined fifteen minutes, one hour and two hours later by the method of Folin and Wu. In forty tests on normal animals the blood sugar increased from 10 to 20 mg per cent over the initial fasting blood sugar level. No rise greater than 35 mg per cent was observed in normal animals, and we have followed Bodansky in accepting this as the maximal normal value.

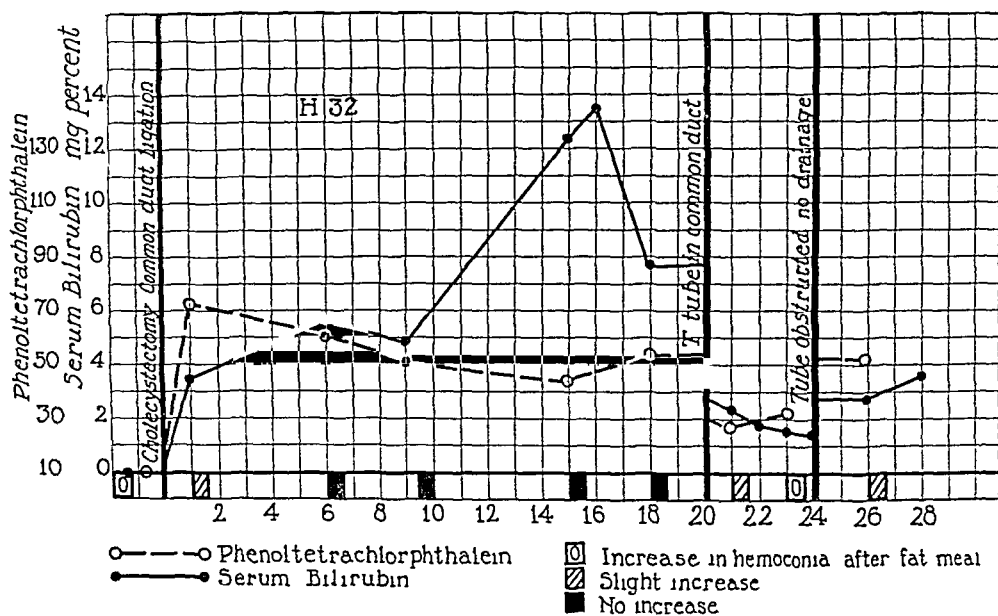


Fig 5 (Dog H32)—Changes in serum bilirubin, phenoltetrachlorophthalein and hemoconia following operative relief of an experimental obstruction to the common bile duct

Postoperative hyperglycemia made the interpretation of fructose tolerance tests difficult the first day after operation. A definite reduction in the carbohydrate tolerance was first observed from the second to the eleventh day after ligation of the common bile duct. There was no significant difference between the two series of experiments. Usually the reaction became positive about the sixth day and remained so until the death of the animal. Rises in the blood sugar of 40 mg per cent or more over the initial fasting level were the rule, and in some instances the initial blood sugar value was doubled. During the latter portion of the postoperative course, fructose tolerance occasionally was normal, but such responses apparently were accidental, since the reaction subsequently became positive. In two instances the fructose tolerance was

² Bodansky, Meyer. Fructose, Glucose and Galactose Tolerance in Dogs, *J Biol Chem* 56:387-393 (June) 1923

estimated on the day of death. In both the fasting blood sugar level was low and no appreciable rise followed the administration of fructose.

The constancy of the changes observed in the fructose tolerance tests following the production of obstructive jaundice may well be interpreted as evidence of disturbance in the glycogenic activity of the liver. It is not necessary to consider the possibility of an associated pancreatic disturbance or diabetes mellitus in experimental animals, as it is in patients with obstructive jaundice. At the same time, the changes in this test cannot be interpreted as absolutely specific. In certain normal animals whose blood sugar during fasting was low, a rise of more than 35 mg followed the ingestion of fructose. Such tests are difficult of evaluation since a definite hyperglycemia did not develop. Also, both in fasting animals and in those on a ketogenic diet, occasional positive results were obtained with maximal blood sugar values considerably above the normal limits.

Blood Sugar Level—The rôle of the liver in the maintenance of the blood sugar level has been fully established by many investigators. In these experiments hyperglycemia was frequently observed on the day following operation, and it was ascribed to the operative procedure. After the induction of obstruction and the development of icterus, the blood sugar level during fasting usually was within the normal limits. In some animals a moderate terminal hypoglycemia was noted, a finding in accord with those of Isaac,³ Williamson and Mann⁴ and others, who made this observation in other forms of injury to the liver, especially in experimental phosphorus and chloroform poisoning.

Nitrogen Partition in the Blood—Complete studies of the nonprotein nitrogenous constituents of the blood were made on each test day. No characteristic differences were noted between the results in the two series of experiments. The blood urea revealed the most consistent and characteristic changes of all the nitrogenous constituents studied. In general there was a definite fall following the development of jaundice. The decrease involved both the relative and absolute values for urea while the total nonprotein nitrogen was less affected. This is well shown in Table 1. Shortly before death a sharp rise in the blood urea was noted as an antemortem phenomenon. These changes in the blood urea are of extreme interest, for Morgulis and Edwards⁵ found that fasting alone

3 Isaac, S. Theoretisches und Klinisches zur Stellung der Lavulose im Stoffwechsel, *Med Klin* **2** 1207-1210, 1920.

4 Williamson, C. S., and Mann, F. C. Studies on the Physiology of the Liver, V, The Hepatic Factor in Chloroform and Phosphorus Poisoning, *Am J Physiol* **65** 267-276 (July) 1923.

5 Morgulis, Sergius, and Edwards, A. C. Chemical Changes in the Blood During Fasting and Subsequent Refeeding, Experiments on Dogs, *Am J Physiol* **68** 477-498 (May) 1924.

caused an increase in the blood urea. Yanagi⁶ and others have reported that the blood urea is decreased in patients with marked jaundice. Rowntree, Marshall and Chesney⁷ showed a decrease in the percentage of the urea to total nonprotein nitrogen, and Bollman, Mann and Magath⁸ have demonstrated that hepatectomy produces marked interference with the formation of urea in the body as evidenced by low values for the blood urea. Nevertheless, it should be pointed out that, while the blood urea in general is decreased following the production of experimental jaundice, the values still fall within the lower limits of normal.

In only two instances did determinable quantities of a substance giving the color reactions of uric acid appear in the blood. Both instances represented isolated observations apparently without general significance. This again serves to emphasize the difference between the reactions in these animals and in those in which the liver has been completely removed, in whose blood Mann has emphasized the regular appearance of uric acid.

Bile Pigments—Bilirubin is not a normal constituent of dog's blood, or at least it is not present in appreciable quantities. From the standpoint of renal excretion it may be considered a non-threshold substance and any free pigment in the plasma is promptly excreted in the urine. With the production of obstructive jaundice the usual pathway of excretion is blocked and bilirubin appears in the blood in amounts greater than the kidneys can excrete. On the appearance of bilirubin in the blood stream the serum first exhibits an indirect van den Bergh⁹ reaction. This reaction later becomes diphasic and finally direct, as the amount of pigment increases.

Only traces of bilirubin were found in the blood twenty-four hours after ligation of the common duct. The van den Bergh reaction usually became direct after from forty-eight to seventy-two hours at which time the serum contained from 2 to 4 mg per cent of bilirubin.

Clinical evidences of obstruction developed more slowly, and frank icterus usually did not appear until the third or fourth day after operation. Bile pigments were not found in the urine before the second day. In this series of animals, the amount of bile retention increased pro-

6 Yanagi, K. A New Colorimetric Method for the Determination of Urea with Urease, *J. A. M. A.* **82** 1169-1171 (April 12) 1924.

7 Rowntree, L. G., Marshall, E. K., Jr., and Chesney, A. M. Studies in Liver Function, *Tr. A. Am. Phys.* **29** 586-625, 1914.

8 Bollman, J. L., Mann, F. C., and Magath, T. B. Studies on the Physiology of the Liver, VIII, Effect of Total Removal of the Liver on the Formation of Urea, *Am. J. Physiol.* **69** 371-392 (July) 1924.

9 Van den Bergh, A. A. H. *Der Gallenfarbstoff im Blute*, Leiden, van Doesburgh, 1918, 8.

gressively until the second or third week, thereafter the serum bilirubin values were constant, aside from slight daily fluctuations

In animals with cholecystectomy, besides ligation of the common duct, jaundice developed much more rapidly than in those with simple obstruction. In two experiments an indirect van den Bergh reaction was first obtained thirty minutes after ligation of the common duct. This reaction later became diphasic. One and one-half hours after ligation of the common duct and removal of the gallbladder, the direct reaction was obtained. The serum at this time contained 0.3 mg per cent of bilirubin. At the end of four hours, the serum bilirubin was 2.2 mg per cent. Twenty-four hours after operation the serum contained from 3 to 5 mg per cent of bilirubin. The urine contained large amounts of bile pigments, and the sclerae and mucous membranes usually had a definite icteric tinge. The amounts of bilirubin in the serum of the animals in

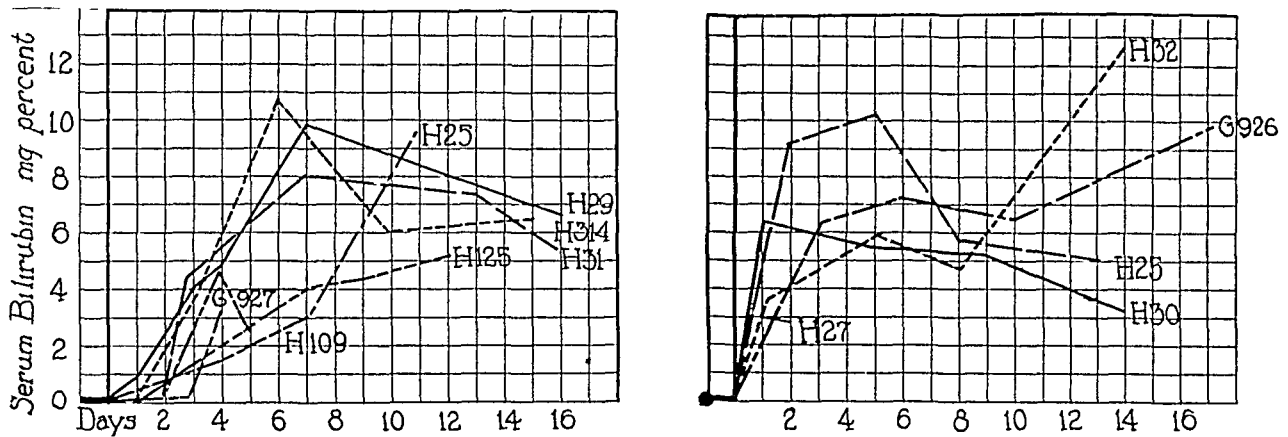


Fig 6—Effect of presence or absence of the gallbladder on changes in serum bilirubin following ligation of common bile duct. Left—without cholecystectomy, right—with cholecystectomy.

this group usually rose steadily during the first week, when a transient decrease occurred, with a subsequent rise to the final level (Fig 6).

The clinical evidence of icterus increased somewhat as the obstruction continued, although the serum bilirubin was fairly constant during the latter portion of the dog's life. During this period there was little, if any, difference between the animals of the two series of experiments. In both groups the serum bilirubin varied between 5 and 10 mg per cent on the average, and no rise greater than 13.5 mg per cent was noted. In this respect the experimental observations differ from the clinical, for while the serum bilirubin was markedly increased in these dogs, it did not approach the excessive amounts occasionally observed in jaundiced patients.

Fasting alone may occasionally cause the appearance of bilirubin in the urine of normal dogs. However, in none of the control animals was it possible to demonstrate the presence of a bilirubinemia.

The Hemoconia Test of Brulé—The rôle of bile acids in fat metabolism has been discussed at length by Brulé,¹⁰ who considers these acids essential for the absorption of fat from the intestines. The estimation of ultramicroscopic blood fat particles (hemoconia) after a fat meal was carried out according to his method in two animals. Betoie operation both showed the normal increase of hemoconia over the control specimen taken during fasting. When biliary obstruction was well established no increase in hemoconia was noted in the ten tests performed on these two animals. Connection between the bile passages and the intestine was reestablished in one (Experiment 3). Thereafter the blood fat particles increased normally following the fat meal. A few days later the tube became obstructed and the test again became positive. These findings are in keeping with the views of Brulé, as he asserts that the saturation of intestinal mucosa with bile pigments may be sufficient to give a positive test for stercobilin in the stool even in the presence of well established obstruction. For this reason it would appear that the test for hemoconia may be somewhat more accurate than the simple stool examination for bile.

Coagulation Factors—The delay in the coagulation time of the blood is a well known clinical phenomenon in cases of obstructive jaundice. It has recently been studied by Walters,¹¹ who has given calcium chlorid intravenously to reduce the coagulation time and thus combat the tendency to hemorrhage following surgical procedures in such cases. The coagulation and calcium time of the venous blood was studied in all animals by the method of Lee and White,¹² who have pointed out that both the coagulation time and the calcium time were greatly prolonged in the presence of demonstrable icterus and remained so until death. The normal coagulation time of dog blood by this method varies between four and seven minutes.

Following the production of obstruction, the usual coagulation time varied between eight and twelve minutes, although the maximal value was fifteen minutes. In general there was a fairly close parallelism between the degree of bile retention and the coagulation time, although in some animals a spontaneous reduction in the coagulation time was noted late in the progress of the condition. The total calcium content of the serum did not vary from the normal following the development of jaundice.

10 Brulé, Marcel. *Recherches sur les ictères les retentions biliaires par insuffisance hépatique*, Paris, Masson et Cie, 1922, **13**.

11 Walters, Waltman. *Preoperative Preparation of Patients with Obstructive Jaundice*, Surg., Gynec. & Obst. **33** 651-656 (Dec.) 1921.

12 Lee, R. I., and White, P. D. *A Clinical Study of the Coagulation Time of Blood*, Am. J. M. Sc. **145** 495-503, 1913.

Blood fibrin determinations were made by the method of Foster and Whipple¹³. A sharp postoperative rise was noted in all animals with a gradual return to normal for several days. Thereafter values approaching the upper limits of normal were the rule, occasional high values were observed, but in no instance was there a definite decrease in the fibrin. No direct relationship between the coagulation time and the fibrin content of the blood was observed.

Fragility Studies—The resistance of red blood corpuscles to hypotonic salt solutions, measured by the method of Giffin and Sanford,¹⁴ was increased materially in two animals after the induction of biliary obstruction. Hemolysis in normal animals began in solutions of about 0.44 per cent, and was complete in solutions of from 0.3 to 0.32 per cent. In

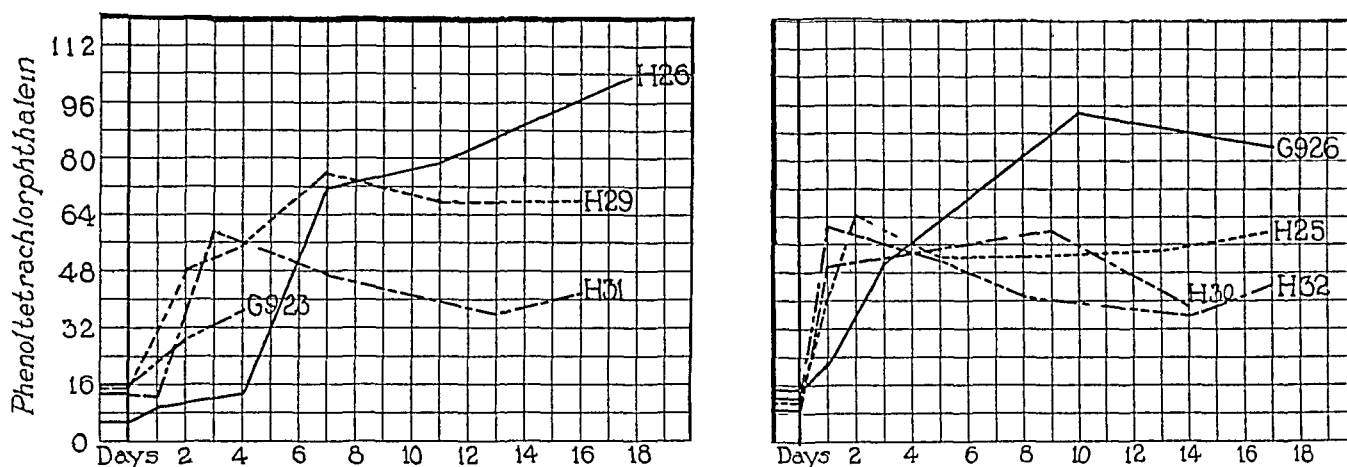


Fig. 7—Effect of presence or absence of the gallbladder on development of phenoltetrachlorophthalein retention after ligation of common bile duct. Left without cholecystectomy, right with cholecystectomy.

the presence of bilirubinemia, dilutions as low as from 0.24 to 0.26 per cent were required for complete hemolysis. This decreased tendency to hemolysis after the onset of jaundice was further evidenced by the appearance of the serum. Before operation it was difficult to obtain blood serum entirely free from hemoglobin, but as soon as the bilirubinemia was well advanced, perfectly clear specimens were the rule. Shortly before death, however, hemolysis was often noted.

Anemia was not a striking feature in any instance although there was a steady fall in the erythrocytes, as measured by the hematocrit, and the hemoglobin showed a slight decline. These findings were to be expected in view of the repeated bleedings to which the animals were subjected.

13 Foster, D. P., and Whipple, G. H. Blood Fibrin Studies, IV, Fibrin Values Influenced by Cell Injury, Inflammation, Intoxication, Liver Injury and the Eck Fistula, *Am J Physiol* **58**:407-431 (Jan.) 1921.

14 Giffin, H. Z., and Sanford, A. H. Clinical Observations Concerning the Fragility of Erythrocytes, *J. Lab. & Clin. Med.* **4**:465-478 (May) 1919.

The Widal Hemoclastic Crisis—Widal¹⁵ has described a postalimentary leukopenia as one phase of what he terms "the hemoclastic crisis." He considers this leukopenia after a protein meal as evidence of hepatic insufficiency. The test was performed on three dogs with jaundice of long duration. The animals showed changes in the other laboratory tests, but the changes in the hemoclastic crisis were not characteristic (Table 4). The test was performed on two other animals before and after operation. Following the obstruction to the common duct, the two dogs showed a slight, but not characteristic leukopenia. All the animals with obstruction showed a high initial leukocyte count with considerable variation in successive counts, the variations made it difficult to ascribe small changes to the effect of the protein meal, and the interpretation of the tests was correspondingly difficult. Our results have led us to question the practical value of this test, at least in experimental work such as this.

TABLE 4—Widal Hemoclastic Crisis

Dog	Duration of Obstruction, Days	Leukocyte Count				
		Fasting	Time After Taking Milk			
			0.5 Hour	1 Hour	1.5 Hours	2 Hours
G 926	14	8,800	7,200	7,600	10,200	11,100
H 26	12	15,900	11,600	15,500	19,800	20,500
H 25	12	20,800	21,600	27,400	32,600	30,000
H 32	0	10,500	10,200	13,600	16,300	19,800
	2	17,400	21,500	23,200	18,800	26,600
	7	19,200	18,600	18,600	19,200	15,200
	15	38,000	33,500	33,300	41,800	37,900
H 31	0	11,500	9,900	11,300	11,800	13,500
	3	21,900	21,700	20,700	21,800	20,400
	10	51,800	48,800	53,000	47,000	55,700

Phenoltetrachlorophthalein—The original test of Rowntree, Hurwitz and Bloomfield¹⁶ could not be applied in the study of obstructive jaundice as it was based on the measurement of the amount of dye excreted in the stools. Rosenthal¹⁷ has since modified the test by substituting the measurement of the degree of the retention of dye in the blood for the study of the excretion in the bile. He found that phenoltetrachlorophthalein retention did not occur in experimental obstruction of the common duct. However, his observations on animals were confined to the first three days after the production of biliary obstruction.

15 Widal, Fernand, Abram, Pierre, and Iancovescu, N. L'épreuve de l'hémoclasie digestive dans l'étude de l'insuffisance hépatique, *Presse med* 2 893-899, 1920.

16 Rowntree, L. G., Hurwitz, S. H., and Bloomfield, A. L. An Experimental and Clinical Study of the Value of Phenoltetrachlorophthalein as a Test for Hepatic Function, *Bull. Johns Hopkins Hosp.* 24 327-342, 1913.

17 Rosenthal, S. M. An Improved Method for Using Phenoltetrachlorophthalein as a Liver Function Test, *J. Pharm. & Exper. Therap.* 19 385-391 (June) 1922.

Our animals were observed for a longer period and definite changes associated with the development of icterus were present.

On account of the rapid disappearance of the dye from dog's blood we used a dose of 10 mg. for each kilogram of body weight twice the amount used for patients. In forty determinations on thirty-five normal animals we found that the greater part of the dye disappeared in fifteen minutes the usual readings being between 6 and 8 per cent after five minutes 3 and 5 per cent after ten minutes and 3 per cent or less after fifteen minutes. Traces of dye were found in blood specimens drawn one hour after its administration but the two-hour specimens contained no dye. In normal animals an average of less than 1 mg. of dye was found in two-hour urine specimens.

We have compared the results of different tests on the basis of the sum of the dye readings obtained in the five specimens of blood taken in each test that is five ten fifteen sixty and 120 minutes after the injection of the dye. In normal animals the total varies between ten and twenty with an average of sixteen. This is an arbitrary method of comparison but has been the most satisfactory for routine use since it is more convenient than the area method of Lamson¹⁸ and is a more sensitive index than the individual test used clinically by us.

No retention of phenotetrachlorophthalein in the blood stream of the dogs was observed until the second or third day following the ligation of the common bile duct. The development of distinct dye retention usually coincided with the first definite appearance of bile in the blood, both occurring from forty-eight to seventy-two hours after operation. Thereafter the amount of retention gradually increased the maximum amount being obtained from the second to the fourth week. At this time the sum of the readings varied between 40 and 60 per cent. on the average while as much as 25 per cent. of the dye injected was present in the serum two hours afterwards. In general a gradually increasing serum bilirubin and dye retention were found the two running an approximately parallel course until the death of the animal.

If cholecystectomy and ligation of the common bile duct were performed dye retention was found twenty-four hours after operation. Here too a close parallelism with the amount of serum bilirubin was observed the direct van den Bergh reaction and distinct dye retention appearing at the same time (Fig. 7). During the period immediately following operation these animals were in general much more affected by their biliary obstruction than those in the first group. Tests made at the end of the first week and during the second week usually showed a fall in serum bilirubin and a decrease in the degree of dye retention.

18 Lamson P. D., Gardner G. H., Gustafson R. K., Maize E. D., McLearn A. J. and Wells H. S. The Pharmacology and Toxicology of Carbon Tetrachloride. *J. Pharm. & Exper. Therap.* 22:215-288 (No. 1) 1923.

Later a secondary rise in both bilirubin and phenoltetrachlorophthalein retention was observed, the curve tending to remain stationary after the third week. The animals in this group lived somewhat longer and were in better condition than those in the first group, once they had become adjusted to their altered conditions and the effect of the bile retention. The amount of dye in the two-hour specimens of urine, in general, ran parallel to the degree of dye retention in the blood.

COMMENT

The morphologic changes in the liver due to long standing biliary obstruction and resultant jaundice are well known. The characteristic pathologic changes recently have been described in detail by Ogata,¹⁹ MacCallum,²⁰ Bell,²¹ Flynn²² and others. No less definite are the functional disturbances evidenced by some of the tests studied by us. Certain tests failed to show clean cut changes due to the production of obstruction. This may imply either that such tests are not specific, or that the pathologic disturbances did not produce functional alterations sufficient to permit of their demonstration by these tests.

While it is known that the coagulation time of the blood is prolonged in cases of obstructive jaundice, the physiologic mechanism determining this delay is not known. The fibrin and the total calcium content of the serum are not deficient. The Widal hemoclastic crisis is not well adapted for use under the experimental conditions in our series, and the results did not correspond with the changes in the other tests. The Brule hemoconia test, on the other hand, shows the presence or absence of bile salts in the intestines, and while it indicates obstruction in the biliary passages, it does not refer directly to the activity of the liver.

Theoretically, the most desirable test for the functional efficiency of the liver would be one that would indicate disturbances in the metabolic activity of that organ. Apart from the difficulty of securing a test specifically measuring the participation of the liver in protein or carbohydrate metabolism, one must also consider the large factor of safety present under normal conditions. The greater portion of the organ may be experimentally removed or destroyed without interfering with the well-being of the animal.

19 Ogata, Tomosaburo. *Beiträge zur experimentell erzeugten Lebercirrhose und zur Pathogenese des Ikterus mit spezieller Berücksichtigung den Gallenkapillaren bei der Unterbindung des Ductus choledochus und der Ikterogenvergiftung*, Beitr z path Anat u z allg Path **55** 236-314, 1913.

20 MacCallum, W. G. *A Textbook of Pathology*, Ed 2, Philadelphia, W. B. Saunders Company, 1920, p. 330.

21 Bell, L. P. *A Study of the Pathologic Conditions Encountered During and Following the Relief of Experimental Obstructive Jaundice Compared with Similar Pathologic Conditions in Man*, Papers Mayo Foundation and University of Minnesota Medical School, 1921-1922, **2** 24-32.

22 Flynn, M. R. Personal communication to the authors.

The decrease in the blood urea following the experimental production of biliary obstruction is definite though not marked. In view of the experiments of Bollman, Mann and Magath, these changes may be considered significant although they are not sufficiently well marked to be clinically applicable.

In cases of toxic injury from phosphorus, chloroform or hydrazin, changes in carbohydrate tolerance parallel the apparent response of the experimental animal. In obstructive jaundice similar changes in the fructose tolerance are obtained, indicating a failure of the organism to assimilate this form of carbohydrate. We believe, therefore, that fructose tolerance tests may be of value in experimental studies such as this, although we have pointed out elsewhere the difficulties in their clinical use.

The changes in the bilirubin content of the serum after the induction of jaundice are characteristic. Emphasis should be placed on the differences noted in the two groups of animals, the development of jaundice being delayed in those with an intact gallbladder. Mann and Bollman have previously pointed out the more rapid development of bilirubinemia in dogs following cholecystectomy, ligation of the cystic duct, or cholecystitis of chemical origin. With Rous and McMaster²³ they have shown that an intact gallbladder has the power of greatly concentrating the bile; this physiologic function of the organ suffices to delay the development of any increase in intraductal pressure and also postpone toxic hepatic injury due to bile retention.²⁴ On the other hand, removal of the gallbladder at the time of the ligation of the common duct prevents this concentration of the bile, the pressure within the hepatic duct rapidly rises, and there is a resorption of bile with a consequent rapid development of bilirubinemia and clinical icterus. The quantitative determination of the serum bilirubin by the method of van den Bergh is of the greatest value in the demonstration and study of these changes.

Much is still to be learned concerning the fate of phenoltetrachlorophthalein after its intravenous injection. If there is obstruction the possibility of its accumulation in the biliary passages with subsequent resorption must be considered, although all the dye leaves the blood stream within a few hours, regardless of the state of the liver. No dye was found in the bile passages of these animals, although they had received repeated doses during the period of their obstruction. Small amounts of phenoltetrachlorophthalein are excreted in the urine, but the greater portion of the dye retained in the organism must have been

²³ Rous, Peyton, and McMaster, P. D. The Concentrating Activity of the Gallbladder, *J. Exper. Med.* **34** 47-73 (July) 1921.

²⁴ Mann, F. C. A Physiologic Consideration of the Gallbladder, *J. A. M. A.* **83** 829-832 (Sept. 13) 1924. Mann, F. C., and Bollman, J. L. The Relation of the Gallbladder to the Development of Jaundice Following Obstruction of the Common Bile Duct, *J. Lab. & Clin. Med.* **10** 540-543, 1925.

destroyed, as there was no evidence of its accumulation in the tissues Kendall ²⁵ has shown that the closely allied phenolsulphonephthalein may be destroyed in this way. We have found that phenoltetrachlorophthalein is rapidly destroyed when incubated with ground liver or muscle. It is probable that a part of the phenoltetrachlorophthalein is destroyed in the body and that organs other than the liver may remove some of the dye from the blood stream. These factors are of minor importance, however, and do not militate against the use of the test, for the deviations from normal observed in animals with obstructive jaundice are characteristic and striking.

Following the production of biliary obstruction there is a close parallelism in the changes in the phenoltetrachlorophthalein test and the increase in the serum bilirubin. If the gallbladder is intact, a departure from the normal is not observed for a period of from forty-eight to seventy-two hours. Dye retention first appears with the development of a bilirubinemia. Thereafter the two tests show corresponding variations. If the gallbladder is removed at the time the common duct is ligated, dye retention will appear within twenty-four hours, coinciding with the early development of jaundice in animals of this group. The changes in the degree of dye retention during the initial period of obstruction apparently are directly determined by the extent of the bile retention. Since this is determined by the presence or absence of the gallbladder, we believe that the concentrating activity of this organ has only an indirect effect on the excretion of the dye.

Following the operative relief of the obstruction and reestablishment of biliary drainage, there is a decrease in the amount of bilirubin in the serum and the degree of dye retention (Experiment 3). While the changes are not so striking as those seen clinically after the relief of common duct obstruction, they nevertheless serve to emphasize further the close correlation in jaundice between the degree of bile retention and the retention of phenoltetrachlorophthalein. It is difficult to escape the conclusion that the two phenomena are directly related, and that dye retention is an index to the toxic effect of the retained bile.

Conclusions regarding the physiologic activity of the liver certainly should not be based solely on studies such as this. The changes indicated by certain hepatic functional tests in experimental obstructive jaundice appear to be significant, especially since they are of aid in determining the relative value of the various tests under controlled conditions and furnish an experimental background for the interpretation of the deviations observed clinically.

25 Kendall, E. C. The Fate of Phenolsulphonephthalein When Injected Into the Animal Organism, Factors Other Than the Kidney Influencing Its "Retention", Preliminary Report, *J. A. M. A.* 68:343-345 (Feb. 3) 1917.

SUMMARY

A comparative study was made of certain tests for hepatic function in experimental obstructive jaundice. The changes observed in animals with simple ligation of the common duct were contrasted with those observed in animals with cholecystectomy and ligation of the common duct. In the presence of the gallbladder, the intoxication was less severe and the onset of icterus delayed. The development of jaundice did not cause changes in the blood sugar level during fasting, although fructose tolerance tests became positive, usually after the sixth day. There was usually a fall in the blood urea level, although terminal renal insufficiency later caused an antemortem rise in the blood urea. The serum bilirubin, determined by the quantitative method of van den Bergh, afforded a simple and accurate means of studying the degree of bile retention and the progress of the icterus. The coagulation time of the blood was prolonged, although the fibrin content was increased and the serum calcium was not changed. The phenoltetrachlorophthalein test in this condition showed dye retention in the blood stream that closely paralleled the degree of bile retention.

Book Reviews

PRACTICAL LECTURES DELIVERED UNDER THE AUSPICES OF THE MEDICAL SOCIETY OF THE COUNTY OF KINGS, BROOKLYN, NEW YORK (1923-1924 Series)
Pp 484, 135 illustrations Price, \$5.50 New York Paul B Hoeber, 1925

The centennial of the Medical Society of the County of Kings, Brooklyn, New York, was marked by a series of public lectures on practical subjects for general practitioners. These lectures on a wide variety of subjects make up the subject matter of this volume, which is profusely illustrated. The lecturers were carefully selected and the lectures are of uniform excellence.

TUMORS OF THE SPINAL CORD THE SYMPTOMS OF IRRITATION AND COMPRESSION OF THE SPINAL CORD AND NERVE ROOTS PATHOLOGY, SYMPTOMATOLOGY, DIAGNOSIS AND TREATMENT By CHARLES A. ELSBER, M.D., Professor of Neurological Surgery, Columbia University, Consultant in Neurological Surgery, Presbyterian Hospital, Attending Surgeon, Mount Sinai Hospital and Neurological Institute, New York City Pp 421, with 354 illustrations Price, \$10.00 New York Paul B Hoeber, 1925

This is a monograph along large lines. Like those by Cushing on "The Pituitary Body" and "Tumors of the Nervous Acusticus," and by Frazier on "Surgery of the Spine and Spinal Cord," it sets another landmark in the advanced development of neurosurgery in America. The bulk of the book is made up of eighty-one case histories, arranged topographically and profusely illustrated with pictures of gross and microscopic lesions, sensory charts, roentgenograms and steps in the operative technic. Then follow chapters discussing the symptomatology, cerebrospinal fluid changes, pathology, diagnosis and treatment. That a depository of special and highly technical data of this kind is useful to the internist is well stated in the following paragraph from the author's preface:

'To recognize that a pain in the chest is not due to intrathoracic disease but to a spinal root irritation, to distinguish between symptoms due to intra-abdominal disease and root symptoms due to spinal disease, to understand the true significance of a brachial, intercostal or sciatic neuralgia, may be difficult and may tax the diagnostic acumen of the physician. The same statement applies to the differentiation of the symptoms of primary disease of the bladder or prostate gland from the rectal and vesical disturbances that occur in spinal disease. Not so rarely patients have been treated for long periods and been subjected to operative interference for supposed intra-abdominal or pelvic disease when the cause was in the spinal cord or nerve roots.'

RECOVERY RECORD FOR USE IN TUBERCULOSIS By GERALD B. WEBB, M.D., and CHARLES T. RIDER, M.D., Colorado School of Tuberculosis, Colorado Springs, Colo. Second edition Pp 81, with 100 weekly record sheets Price, \$2.00 New York Paul B Hoeber, 1925

This book was first published in 1923, and has been reviewed in this journal; this second edition is a first reprinting and not a revision, as stated by the publishers. The book has been of great aid to convalescent tuberculous patients because of the sound advice given in language an average layman can understand, and because of the excellent weekly progress record sheets for the use of the patients attached.

THE ACTION OF OILS IN THE PRODUCTION OF TUMORS

WITH A DEFINITION OF THE CAUSE OF CANCER²

MONTROSE T BURROWS, M D

AND

CHARLES G JOHNSTON, M D

ST LOUIS

In 1906 B Fischer introduced drops of a saturated solution of scharlach r in olive oil directly under the epidermis of the ears of rabbits. He notes that the epithelial cells grew down to surround these oil droplets. The histologic picture of this growth of the epithelial cells resembled epitheloid cancer in many cases, but these formations always ceased to proliferate and regressed after a time. Reinke introduced drops of ether into the eye of salamanders. A growth of cells was induced by the presence of this substance. He transplanted this new growth to the peritoneal cavity and found it continuing to proliferate for a time. White succeeded in inducing epithelial activity with drops of oleic acid, and Strober and Wacker with sudan III, indol and skatol. Benthun used several such materials, and Bullock and Rohdenberg and others have tried the effect of introducing scharlach r into various internal organs. In each instance these authors noted the same result: an encompassing of the oil with cells, certain grades of degeneration and proliferation, then a gradual regression of the newly formed tissue.

While there seemed little doubt from these experiments, as Wacker and others have pointed out, that certain lipid solvents may excite epithelial growth, there was no evidence that they could excite true cancer¹. On the other hand, it had long been known that certain workers exposed continuously to the action of soot (chimney sweeps²) and the action of other coal tar products are particularly predisposed to develop cancers of the skin, and that these individuals develop their cancers in localities of the skin where the soot or other substances are present frequently over a long period of time. Yamagiwa, appreciating

¹ From the Research Laboratories of the Barnard Free Skin and Cancer Hospital, and the Department of Surgery, Washington University Medical School.

1 Ewing, J. Neoplastic Diseases, Ed 2 Philadelphia, 1922.

2 Potts, P. Surgical Observations, London, 1775.

the significance of the observations of B Fischer and the relation of these observations to the development of cancer in coal tar workers, noticed that the difference between the experiments performed and the development of cancers in men exposed to lipid solvents is that the animals studied received only a single dose while the men developing cancer were often irritated repeatedly by these substances for many years before their cancers appeared

Yamagiwa and Ichikawa³ then undertook the study of the effect of repeated applications of coal tar to certain restricted areas of the skin of the ears of rabbits. They found a piling up and proliferation of the epithelial cells of the skin after each application. Very large papillomas developed in some instances. In others the tissue not only suffered proliferation but also an associated degeneration and ulceration after each application of the tar. Eventually many of these tumors became cancers and metastasized. Since that time many workers have succeeded in producing carcinomas by repeatedly painting coal tar on certain areas of the skin of rats and mice (Woglom and Murray,⁴ Fibiger,⁵ Deelman,⁶ Lipschitz⁷ and others). Other similar substances, such as sudan III in olive oil and paraffin oil, have also given similar results. How these substances act to produce this disease has not, however, been fully explained. Clowes⁸ suggested that these substances may act to initiate growth by dissolving the lipid membrane of the cell. Bullock and Rohdenberg⁹ thought that these substances acted to destroy the cells. They noted that many cells coming in contact with the tar degenerated. They considered that the growth followed this primary death of the cells. Champy¹⁰ has outlined a similar theory. He concludes that the primary action of the tar is a destruction of cells. Other cells then grow to replace the defect from this loss of cells. The cells thus continuously stimulated to regenerate finally acquire the property of independent growth.

3 Yamagiwa, K, and Ichikawa, K. Experimental Studies on the Pathogenesis of Cancer, *J Cancer Res* **3** 1 (Jan) 1918

4 Woglom W H, and Murray, J A. Experimental Tar Cancer in Mice, Seventh Sc Rep Imperial Cancer Res Fund, London, 1921, p 49

5 Fibiger, J. Etat actuel des recherches sur la production experimentale du cancer, les buts de ces recherches et les problemes qui en preunent leur origine, *Acta chir Scandinav* **55** 343, 1922

6 Deelman, H T. Ueber die Histogenesis des Teerkrebses, *Ztschr f Krebsforsch* **19** 125 (Oct) 1922

7 Lipschitz, B. Untersuchungen uber die Entstehung des experimentellen Teercarcinoms der Maus, *Ztschr f Krebsforsch* **21** 50, 1923

8 Clowes G H. Tr XVII Internat Cong Med, London, 1913

9 Bullock, F D, and Rohdenberg, S L. A Study of the Scharlach R Reaction and of Allied Forms of Epithelial Proliferation, *J Med Res* **33** 53, 1915

10 Champy, C, and Vasilin, I. Recherches sur le cancer experimental du goudron, *Bull du Cancer* **12** 111, 1923

Other authors have searched for specific substances in the tar. Several by-products of tar have been isolated and studied. Kennaway¹¹ states that isoprene causes cancers to develop more readily than coal tar. Other substances also have been isolated and studied. Kennaway, in one of his analyses, finds that the viscid tar of horizontal retorts is more active than the tar distilled in vertical ones, and that certain by-products of tar are more active than others.

It must be pointed out here that the more recent and older work on cancer has shown that it may not only be induced by coal tar and other lipid solvents, but also by a number of different conditions and substances, such as roentgen rays, radium, certain animal parasites¹² and bacteria, that it may follow long standing chronic inflammation, atrophy from any cause, congenital tumor and defects, and probably in certain instances other injurious agents. In many of these studies, it has also been shown that these substances are not responsible for the eventual course of this disease but are important only in inducing it. Cancer is evidently not a disease to be classed with the infectious diseases¹³ but is an active, independent growth of body cells. This primary growth is the result of some primary change either in the cell or in the tissue which may be induced by any one of the various substances or conditions mentioned above. When this change is once established, the cells then continue to grow indefinitely at the expense of the organism and independent of the causative agent. While it is true that Yamagiwa and Ichikawa³ and later authors have shown quite definitely that coal tar repeatedly applied to areas of the skin will produce cancer, the cancers once induced have in each instance then proceeded independently of the tar. Many of these have been transplanted repeatedly to other animals and have continued to grow actively long after the tar had completely disappeared. The same is true of the cancers induced by animal parasites. Several years ago, Edwin Smith¹⁴ isolated a bacterium, *B. tumefaciens*, from a cancerous growth in a plant. This organism is easily cultivated in a nutrient medium and will induce cancers when injected into other plants. Recently, Blumenthal, Auler and

11 Kennaway, E. L. Cancer Producing Substances from Isoprene, *J. Path. & Bacteriol.* **27** 233 (July) 1924, On Cancer-Producing Tars and Tar-Fractions, *J. Indust. Hygiene* **5** 462 (April) 1924.

12 Fibiger, K. On Spiroptera Carcinomata and Their Relation to True Malignant Tumors, with Remarks on Cancer Age, *J. Cancer Res.* **4** 367, 1919. Bullock, F. D., and Curtis, M. R. The Experimental Production of Sarcoma of the Liver of Rats, *Tr. New York Path. Soc.* **20** 149, 1920.

13 Burrows, M. T. Is Cancer a True Disease or Merely the Result of a Change in the Organization of the Organism? *Radiology* **4** 407, 1925.

14 Smith, E. F. Studies on the Crown Gall of Plants, Its Relation to Human Cancer, *J. Cancer Res.* **1** 231 (April) 1916.

Paula Meyer¹⁵ and Nuzum¹⁶ have isolated several organisms from human cancers. One of the organisms isolated by the German authors resembled *B. tumefaciens*. Others were cocci. They found *B. tumefaciens* capable of inducing cancers in both plants and lower animals. The other organisms they have isolated have produced cancers in lower animals.

All malignant tumors do not contain such organisms. Even the most malignant tumors are often free from them. We have sought in vain to demonstrate bacteria in a rat sarcoma now being propagated in the laboratory. Jensen,¹⁷ in studying the plant cancers induced by *B. tumefaciens*, has found that the bacteria are probably unimportant in doing more than inducing the cancer as coal tar, the roentgen ray and radium induce it. He found that these bacteria may disappear in these tumors after a time although the tumor itself may be growing as actively as before. There is no evidence that the growth of the cancer induced by the action of roentgen ray and radium is due to the continuous action of the rays but to changes in the cells or tissues primarily induced by them. The same is true for the cancers of the stomach induced by the spiroptera¹⁸ and the sarcomas noted by Bullock and Curtis¹⁹ to form about the larva of the cat tapeworm in the liver of rats. The cancers proceed but these organisms always disappear.

The problem to be solved in the study of the action of coal tar and other lipid solvents on the tissue is the nature of this change induced either in the cell or in the environment that allows the cells to grow independently. This problem cannot be solved through a study of the physical and chemical properties of these lipid solvents alone, but will become understood only through a broadening of our knowledge of those general conditions which control cellular activity in the normal organism. The organism is a mass of cells. It has come about through a definitely regulated growth of the egg cell and the formation of the different types of cells. This organism matures and functions through a gradual change in the cells so that they use their energy for function rather than for growth. This organism maintains itself for a given period of time in that enough energy is used for growth to take care of the destruction suffered by the cells in their function.

There has never been any evidence to show, however, that the functioning body cells may not under the proper conditions utilize their

15 Blumenthal, Ferdinand, Auler, H., and Meyer, Paula. Ueber das Vorkommen Neoplastischer Bakterien in Menschlichen Krebsgeschwulsten, Ztschr f Krebsforsch **21** 26, 1924.

16 Nuzum, J. W. Experimental Primary Epithelioma from Micrococcus, Surg, Gynec & Obst **40** 343 (March) 1925.

17 Jensen, cited by Blumenthal (Footnote 15).

18 Fibiger (Footnote 12, first reference).

19 Bullock and Curtis (Footnote 12, second reference).

entire energy for growth. It is evident that in such a colony of cells as the body of man, in which the cells as a mass are using their energy for function, if one cell breaks away from the conditions that force it to function and acquires the property of independent growth, it must soon destroy its neighbors weakened by their functional burdens.

These recent researches have, therefore made the study of the action of oils in the production of cancer and the whole cancer problem one of the most fascinating and most hopeful for the development of broad biologic generalizations. The cancer problem as it is known today deals with those fundamental processes which regulate the normal growth, function and death of the organism.

Many of the earlier authors had already appreciated that cancer is an independent growth of body cells, a parasitism of one cell or a group of cells on their neighbors forced to work for the whole rather than acquire nutrition and use it for their own development. In attempting to explain it as such, certain of these writers thought that the control exercised by the whole over the growth and function of the cells was associated with permanent irreversible age changes in the physical and chemical structure of the cell. They looked, therefore, at the cancer cell as a cell that had become changed. It is a specially differentiated cell. The cancer cell arising from the skin epithelium differs from the skin in the same general manner as the skin epithelial cells differ from the muscle cells.

There was another group of authors among whom Ribbert²⁰ was preeminent, who took quite a different view. They looked at the active growth in cancer as the result of primary changes in the environment about the cells rather than in the cell itself. Ribbert based his argument on the fact that there is no evidence that the cells of the body lose their power to grow during development or in later life. That wounds heal readily in old persons and that the tissues of these old persons may regenerate prove this fact conclusively. The stopping of growth at maturity is the result of the acquired organization of the whole or the environment about the cells, and is not due to any irreversible age change in the cell.

In further proof for this deduction came the experiments of Driesch,²¹ those of many other students of development, and the more careful analysis of the peculiarities of the cancer cells on which the adherents to the idea of a specially differentiated cancer cell clung. Driesch had noted that when a sea urchin gastrula is bisected, each half will regenerate into a complete sea urchin embryo and adult. These

20 Ribbert, H. Das Karzinom des Menschen, sein Bau, sein Wachstum, seine Entstehung, Bonn, 1911.

21 Driesch, H. Die Localization morphogenetischer Vorgänge, Arch. f. Entwicklungsmech. d. Organ. 8 35, 1899.

bisected halves do not begin to grow at once, however, but only after each has reformed by a shifting of cells into a perfect gastrula of one-half the normal size. The embryonal cells of the gastrula do not grow under all conditions. When the gastrula is bisected, they show only migration. Only when a special form is obtained in the whole does the growth of the cells again intervene. Again many students of cellular growth and cell division have shown that these acts may be initiated by outside means or by changing the environment, and that they are controlled definitely by the environment and not by the cell in the normal organism.²²

The chief arguments that the cancer cell is different from the normal cell are that it has a different shape, it grows independently, it stains differently, its mitotic figures may be irregular and it may contain inclusions. Each one of these peculiarities has now been shown to be easily reproduced by simply changing the environment about any normal cell. The body cell is a fluid system that can be easily molded. Its shape is determined entirely by its outside contacts.²³ Any normal cell growing in the proper environment may take up foreign matter, may divide irregularly as cancer cells divide, and may stain as cancer cells stain. Wilson has shown that a substance that induces the unfertilized egg to develop normally will induce abnormal mitosis in eggs if allowed to act too long or in too great concentrations.²⁴

While Ribbert and the schools proceeding and following him were unable to ascertain the nature of the environment suitable for cells to grow independently, the weight of evidence has always been with them in spite of the fact that a large majority of workers in this field have taken the other view. This flocking of the larger school to the standards of a different type of cells in cancer has not been, evidently, from an analysis of the facts at hand, but from the greater influence of men like Cohnheim and other leaders in pathology. The embryonal theory of cancer was apparently too fascinating. They refused to leave it and admit that cancer cells arising from the epidermis are nothing more than independently growing epidermal cells.

It remained, therefore, to find some means to isolate normal living body cells and study their habits in various kinds of environments. Reverdin had shown that small pieces of skin removed to a wounded surface grow and later attach themselves to the normal organism and aid

22 Wilson, E. B. *The Cell in Development and Heredity*, Ed. 3, New York, p. 1029.

23 Burrows, M. T. *Energy Production and Transformation in Protoplasm as Seen Through a Study of the Mechanisms of Migration and Growth in Body Cells*, *Am. J. Anat.*, to be published.

24 Wilson, E. B. *Experimental Studies in Cytology, a Cytological Study of Artificial Parthenogenesis in Sea-Urchin Eggs*, *Arch. f. Entwicklungsmech. d. Organ.* **12** 529, 1901.

in covering the defect Hertzler studied these skin grafts carefully to find that they take only when they are placed on a surface covered with a layer of clean fibrinous exudate. They grow into this exudate to make firm union with the body later.²⁵ In 1905 Harrison had noted that when the neural tube of young frog embryos is placed in a thin layer of lymph on the surface of a cover glass in a moist air chamber that the cells migrate and nerve fibrils grow from them into the clotted lymph. No actual growth of cells was noted, however, in these cultures by Harrison.²⁶

The senior author, having the opportunity to work with Dr. Hertzler while he was performing his experiments on the healing of wounds in the peritoneum and skin grafting, appreciated at a latter date that the cells which grow first from the Reverdin skin grafts, grow quite independently of the body. Hertzler found that these grafts take only when the wound is covered by a layer of fibrin. In many such cases the grafts are separated from the underlying blood vessels by this fibrinous layer. Since substance diffuses poorly into such fibrinous layers, the cells migrating into the exudate from these fragments must be cut off largely from the body. These cells should grow readily, therefore, when removed to a layer of fibrinous exudate outside the body. In the summer of 1910, under a grant by the Rockefeller Institute, the senior author commenced to study the cultures of Harrison in his laboratory and attempted to develop a method for cultivating the tissues of higher animals. Blood plasma was first substituted for the lymph used by Harrison. In thin layers of plasma suspended from a cover glass into a small moist air chamber, the cells were found to grow readily and actively in many cultures of fragments of chick embryos.²⁷ These cells, it was interesting to note, did not grow in every culture of fragments of the same tissue placed in layers of the same medium, and where growth intervened the cells did not tend to arrange themselves into organ structures peculiar to their growth in the body, but they invaded the medium along the lines of least resistance, exactly as cancer cells grow and invade the organism. It seemed evident, therefore, that the secrets of the problem of cancer lay hidden in the conditions that allowed these cells to grow in this fashion in these cultures, or in the environment that the cultures

25 Hertzler, A. E. *The Peritoneum*, Chapter V, **1**, 1919.

26 Harrison, R. G. *The Outgrowth of the Nerve Fiber as a Mode of Protoplasmic Movement*, *J. Exper. Zool.* **9** 787, 1910.

27 Burrows, M. T. *Culture des Tissus d'embryon de Poulet et spécialement cultures de nerfs de Poulet en dehors d l'organisme*, *Comp. rend. Soc. de biol.* **2** 291, 1910, *The Cultivation of Tissues of the Chick-Embryo Outside the Body*, *J. A. M. A.* **55** 2057, 1910, *The Growth of Tissues of the Chick-Embryo Outside the Animal Body, with Special Reference to the Nervous System*, *J. Exper. Zool.* **10** 63, 1911.

imposed on them. One year later, this author then undertook in the Anatomical Laboratory of Cornell University Medical College, New York City, the problem of the conditions that allowed these cells to grow in the cultures. The medium chosen for this study was plasma prepared from the blood of healthy animals. The plasma was selected as medium because it represented a normal fluid existing everywhere in the normal organism. There was no evidence to show that cancer developed necessarily in an abnormal organism. In fact, transplanted cancer fragments grow best in young, healthy animals.

This study showed that in plasma this active independent growth of cells depends on three factors: (1) an ample supply of oxygen (which is about one third of the oxygen concentration of the air²⁸), (2) a crowding together of the cells in a minimum amount of medium, and (3) a stagnation of this medium. These factors applied not only for the growth of normal cells, but also for the growth of cancer cells *in vitro* (animal and human cancer, carcinomas and sarcomas)²⁹.

Single cells carefully washed of tissue juices will not grow and often fail to show any reaction whatsoever when placed in a layer of pure plasma diluted with salt solution. On the other hand, if a salt solution extract of any actively growing tissue is added to the plasma, then single cells show immediate activity. They migrate, take up fats and proteins, digest these substances, grow actively and divide by mitosis.

In pure plasma, such an active growth will take place only about fragments of tissue. About these fragments, it is proportional to the age of the tissue, to the size of the fragment and cell density of the fragment and inversely proportional to the thickness of the layer of medium or the amount of absorbing medium about the cells. These proportions in relation to the size of the fragment and the thickness of the layer of medium hold only within limits of active diffusion of oxygen. These diffusion limits were measured and found to be from 1 to 2 mm for most tissue fragments and from 0.5 to 0.7 mm for clotted plasma or serum. About fragments of tissue larger than from 1 to 2 mm in diameter, the growth is inhibited by a lack of oxygen to their central parts and toxic products liberated by the autolysis that results. Definite abnormal reactions are noted in cells in the deeper parts of layers of plasma thicker than 0.5 to 0.7 mm.

The relation of the size of the fragments and the thickness of the layer of medium was studied in fragments of tissue ranging from

28 Burrows, M. T. The Oxygen Pressure Necessary for Tissue Activity, *Am J Physiol* **43** 13 (April) 1917.

29 Burrows, M. T. The Tissue Culture as a Physiological Method, *Tr Cong Am Phys & Surg* **9** 77, 1913. *Tissue Culture in Vitro*, Tr XVII Internat Cong Med, Gen Path and Path Anat, London, p 217, 1913. *The Cultivation of Human Cancer Cells in Vitro*, *M Rec* **86** 649, 1914.

1 mm in diameter to a single cell, and in layers of plasma ranging from 0.2 to 0.7 mm in thickness. In this study it was noted that while growth will obtain about fragments of a given tissue 1 mm in diameter in a layer of medium 0.5 mm thick, it will fail about a 0.5 mm thick fragment of the same tissue in the same thickness of the layer of medium, but succeed about this smaller fragment (0.5 mm thick fragment) in a thinner layer of plasma medium, 0.25 mm thick.

Under these conditions of the proper thickness of the layer of medium and the proper size and cell density of the fragment, it is interesting to note that growth does not begin at once, but always after a given latent period. This latent period is shortest about the cellular fragment of actively growing tissues of young embryos and cancer. It increases in length about similar fragments of older and older embryos and is longest about the less cellular, nongrowing fragments of the adult.

As shown in another article²³ this difference in the latent period is not related to the age of the tissue in these cases, but to their immediate growth rate. It is much shorter for the cells of a piece of growing granulation tissue than for those of the normal connective tissue and shorter for the cells of an actively growing cancer than for those of a slower growing one.

It must be pointed out here that not only the growth of the cells is dependent on a proper supply of oxygen, stagnation, cell crowding and a proper minimum amount of medium, but all activity in these cells is dependent on these conditions. The cells in a cellular fragment of tissue first show migration, then growth and finally a complete disintegration or self-digestion³⁰. These various changes take place in regular sequence. Growth is only one phase of a general change induced in them by their environmental surrounding in the culture. Each of these changes begins in the cells of the center of the fragment. It then spreads from this point to involve more and more of the cells toward the periphery. The cells in a 1 mm thick fragment of mesenchyme of a young embryo placed in a layer of plasma 0.5 mm thick show all of these changes. As one decreases the size of such fragments, leaving other conditions unchanged, the amount of each of these changes not only decreases, but they begin to disappear in the reverse order in which they appeared about the larger fragments. In a culture of a medium size fragment self-digestion disappears except in the cells in the center of the fragment and growth becomes very limited. In still smaller fragments migration alone is observed. The cells in the latter cases do

30 This digestion of cells has been called self-digestion to distinguish it from autolysis. It is a digestion that takes place in the presence of oxygen and does not liberate toxic products. Autolysis, as the word is used, is a digestion taking place in the absence of oxygen. Toxic products are liberated in the autolysis of cells.

not disintegrate, but after becoming sufficiently separated they come to a state of complete inactivity. In this state they remain intact but inactive until destroyed by outside means. The cells in fragments of mesenchyme of older embryos and of the connective tissue of adults behave in the same manner. The cells in the sparsely cellular fragments show only migration and then inactivity. In the more cellular fragments growth and self-digestion intervene in proportion to the cell density of the fragments. The chief difference between these fragments of the older tissues and the more actively growing ones is that these changes take place after a much longer latent period.

From these observations it became evident that the life of these cells is something that is determined by their immediate surroundings. It is the result of a reaction between them and their environment. It has been taught that body cells are continuously active. Bayliss points out that there is no evidence for this view.³¹

These cells which had migrated from the fragment and come to rest apparently disproved it. Several such cultures have been kept in the presence of an ample supply of oxygen and in the incubator for as long as six months. After fourteen days the cells became inactive and remained so for the remainder of this entire period. They showed no changes in shape nor changes in their protoplasm. The fat droplets and others contained bodies that remained undisturbed in them during this time. At the end of this period these cells were found able to become active again. They migrated and grew when placed in drops of fresh plasma.³²

The picture of life in these cells isolated from the body assumes, therefore, proportions of much greater simplicity than had been generally suspected from a study of them in the body. While previous students³³ of regeneration had appreciated that growth is regulated by factors other than food and oxygen, the nature of these other factors and their significance seemed impossible until we had found a method for isolating these cells and studying them under known and controllable conditions. Summing up the foregoing conditions necessary for their cellular activity in the culture, it became evident that they may signify that activity in these cells is dependent on the accumulation of some substance or substances formed by the cells but also readily diffusible into plasma. They also indicate that migration, growth and self-digestion may be merely the result of different concentrations of this substance or substances. Cells showing only migration and then complete inactivity had been seen only in culture of the more sparsely cellular fragments of tissue of older embryos and adults. Growth and

31 Bayliss, W. M. *The Principles of General Physiology*, London, 1915.

32 Burrows (Footnote 29, second reference, Fig. 4).

33 Morgan, T. H. *The Physiology of Regeneration*, *J. Exper. Zool.* **3**: 457, 1906.

self-digestion are peculiar to the more cellular fragments of embryonic mesenchyme and cancer. When a large number of cells are crowded into a small amount of medium, all phenomena proceed. When few cells are placed in a relatively large amount of medium, the cells may become inactive after showing only migration.

An attempt was made, therefore, to ascertain whether such a substance or substances accumulated in these tissues during the latent period and accounted for the activities noted in the cells. Fresh fragments of actively growing tissue of embryos and cancer and slower growing tissues of older embryos and adults were extracted with an equal weight of isotonic sodium chlorid solution, and the extracts tested by placing equal parts with equal parts of plasma. The mixture was used as medium for isolated embryonic heart muscle and mesenchyme cells. Fragments of the same tissue left in a culture for from forty-eight to 128 hours were also extracted and the extracts tested in the same manner. In the presence of the extracts of the fresh tissues of the older embryos and adults, the isolated cells suffered little or no change, while the extracts of similar fragments left in the stagnant cultures for several days stimulated an active migration, growth and in some instances self-digestion in these isolated cells. Extracts of fresh tissue of the younger embryos and cancer stimulated immediate activity in these cells.

The cells of the more inactively growing tissues lying in the stagnant culture had evidently accumulated a definitely active substance. Extracts containing this active substance, S , were then carefully studied. In low dilutions (S^1), they were found to have no effect. In medium concentrations (S^2), they stimulated the cells to migrate and engorge themselves with particles of proteins and fat droplets. In high concentrations (S^3), these cells digested these proteins and fats, grew actively and divided by mitosis. In all higher concentrations (S^4), the cells suffered a self-digestion.

The picture of migration followed by growth and then self-digestion was only the result of a gradual accumulation of the S in the stagnant layers of plasma. A few cells had failed to grow in the thicker layers of medium because the proper concentration of the S could not be formed and retained by them. It is soluble in the salt solution and diffuses away into the plasmatic medium. A few cells cannot supply a large enough quantity of this substance to raise its concentration to the growing point in a large bulk of medium. About the less cellular fragments placed in a relatively large bulk of medium, the cells come quickly to rest because the diffusing S comes quickly to a concentration too little for any kind of cell activity. The cells cannot retain this substance. Its concentration is determined not only by the amount formed by the cells but also by the amount removed by the medium.

On account of these facts this substance or substances, *S*, has been named the *archusia*, the driving substance of the cell. It is evidently the energy for life in the cell. In studying its formation it was found that it is formed only in the presence of oxygen and certain food materials, not determined, but easily exhausted from a drop of plasma and renewed by adding fresh plasma or serum. Again, it was noticed that when it is present in the medium, the cells will react normally without oxygen until it is exhausted. They then suffer autolysis³⁴. It is the only substance in the cell whose formation demands oxygen. In its presence digestion and synthesis proceed without oxygen.

The condition necessary for growth in the body cells is probably not different, therefore, from that noted by Wildiers for yeast. Single yeast cells will not grow in a large bulk of medium unless an extract of yeast is added, while a number of yeast cells added to the same quantity of medium begin to grow very actively after a latent period. Wildiers noted that this failure for the single cell to grow is due to the absence of a substance, *bios*, which is contained in the extracts of yeast and appears in the wine casks when a sufficient number of yeast cells are added³⁵.

In the more careful analysis of this phenomenon discovered in body cells, the active substance, the *archusia* (*S*), has been found to be formed by the oxidation of food substances by the cell and shown to act to produce the manifestations of life in the cell, and to act according to its concentration to produce these different manifestations. It is evidently comparable, therefore, to the heat in the steam engine. The heat acts in the engine to produce work in proportion to its concentration. The *archusia* differs from the heat only in that it induces chemical changes in the cell rather than purely physical ones.

In the engine, it is well known that the work performed is as much dependent on the heat conserved for the water as the heat produced in the fire box. Take off the heat jacket of the boiler so that the heat may escape to the outside, the engine stops. It is interesting to note that the same holds for the cells. The body cell has lost or has failed to develop means to retain its energy, the *archusia*. This escapes from it or is not formed by it except when it is placed in a stagnant environment rich in food and oxygen, and in association with other cells also forming it. In recent years a great amount of study has been devoted to determining the amount of oxygen adsorbed in the body and the carbon dioxide and water eliminated. It is interesting that these studies have yielded little of immediate practical importance. From the

34 Burrows, M. T. The Reserve Energy of Actively Growing Embryonic Tissues, *Proc Soc Exper Biol & Med* **18** 133, 1921.

35 Wildiers, E. Nouvelle substance indispensable au developpement de la levure, *La Cellule* **18** 311, 1901.

studies cited above, it becomes evident that the activity of the body is not alone dependent on the energy produced but also on the energy conserved

FUNCTION AND DEVELOPMENT OF DIFFERENT TYPES
OF CELLS OF BODY

In the first cultures of chick embryos, active rhythmic contraction of the heart muscle cells was also observed in the fragments and whole hearts transplanted to the cultures²⁷ It was interesting to note that these heart muscle cells which had been contracting in the fragment lost this ability to contract as they migrated from the fragment into the medium of most cultures In the medium they assumed the shape, grew and divided like simple mesenchyme or sarcoma cells It became evident, therefore, at this early time that whether these cells are to grow, divide and invade the medium or contract rhythmically depends probably on their immediate surroundings³⁶ In these cultures of embryonic tissue, it was interesting to note, however, that their tendency was to revert from a functional to a growing state Only the cells in the more central regions of the fragments continued to contract, while those in the periphery stopped this act and invaded the medium as fast as the cells outside them moved away

The cells in fragments of embryonic striated muscle from the limbs and back also contract rhythmically in the cultures The contractions are exactly like those of the heart muscle These muscle cells in contact with the medium also quickly revert to cells indistinguishable from the simple mesenchyme or sarcoma cells Under proper conditions they may grow actively and divide by mitosis, or they may migrate as long spindle shaped cells or spread from the tissue as an open syncytial network

While in the great majority of cultures, there is no tendency for these cells which migrate from the fragment to revert and develop function again, in an article³⁷ published in 1912, one cell and an open syncytium were noted to undergo this change Since that time eight more such isolated contracting cells and contracting syncytia of cells have been observed in eight cultures out of several thousand studied with this and other ends in view These cells when cut from the circulation of the body and placed in the stagnant culture accumulate archusia about them and thus tend always to revert to a state of growth rather than function

36 Burrows, M T An Attempted Analysis of Growth, *Anat Rec* **9**, Paper 11, 1915, *The Tissue Culture in Cancer*, Tr Second Pan Am Sc Cong Washington, Section 8, Part 2, p 494, 1915

37 Burrows, M T Rhythmische Kontraktionen der isolierten Hertzmuskelzelle ausserhalb des Organismus, *Munchen med Wchnschr*, No 27, p 1, 1912, *Science* **36** 90, 1912

In the cultures where one places a fragment of embryonic muscle tissue from 0.5 to 1 mm thick, the central parts of these fragments suffer a self-digestion³⁸ from an overabundance of the archusia. A liquid substance is liberated by this digestion. This liquid flows out over the surface of the layer of medium to form a tough film. This film is not only rich in food material but also in archusia. The heart muscle cells entering it flatten out to take an otherwise spindle or irregular shape, grow actively and divide by mitosis. The fragment is also rich in accumulated archusia at this state.²³

Noting the latter facts, it had become of interest to see if the environment played any rôle in determining this rhythm in such isolated rhythmically contracting cells. While it is true that much has been written about the mechanism of muscular contraction, nothing is known except certain isolated facts.

1. Bernstein noted several years ago that the energy of muscular contraction has a negative rather than a positive temperature coefficient. Only surface energy can have a negative coefficient in muscle.

2. Fletcher and Hopkins had noted that lactic acid is liberated at the contraction phase and that oxygen is absorbed only during relaxation.

3. Electrical responses to contraction and the heat changes in muscle have also been carefully measured.

It was interesting in observing these contracting heart muscle cells to note that they occupied a peculiar position in the medium. They were not at the surface of the medium nor in the clot but stretched through a serum cavity between the fragment or the surface film and the ends of the bands of fibrin. One end of these cells was in contact with an area rich in archusia, the other end was in contact with bands of fibrin extending into the open medium. In this medium the archusia diffuses readily. This end in contact with the fibrin must have a very low archusia value.

These cells had gained this position through adhering to the clot, which had loosened from other parts of the side of the fragment and contracted away from it. They had thus become stretched between the fragment and the clot. In other cultures isolated cells were dragged out by the same means so that they became stretched between the surface film and the ends of fibrin fibrilli in the clot.

In studying the action of archusia on the cells, it was found that it acts to liberate another substance, the *eiigusia*. This substance decreases the surface tension of cells. Such a decrease in surface tension is associated with electrical changes. This decrease in surface tension causes the

38 Burrows, M. T., Burns, J. E., and Suzuki, Y. Studies on the Growth of Cells. The Cultivation of Bladder and Prostatic Tumors Outside the Body, *J. Urol.* 13 (Feb.) 1917.

cell to stretch in the direction of its lowest surface tension. In these cells this decrease in surface tension can take place only at the end of the cell in contact with the fragment or the surface film. Under the influence of this decrease in surface tension at one end, these cells must suffer one of three kind of changes: (1) a breaking loose from the fibrin, (2) a tearing of the cell in the middle, or (3) a breaking down of the surface tension lowering substance or the substance that adsorbs it. In the great majority of cultures in which fibrin contracts as described above, the cell quickly breaks loose from the clot. None of them break in the middle. In a few out of the several thousand rhythmicity resulted. These cells contract as the result of a periodic reduction in their surface tension followed in each instance by a breakdown of this surface tension lowering substance or the substance adsorbing it. Lactic acid is liberated. Lactic acid increases the surface tension of the cell or makes it shorten. The ergusia reforming decreases the surface tension or makes the cell lengthen. Such a breakdown is associated with an electric current passing from one end of the cell to the other. It will result always when any simple system like these cells is polarized as the contracting heart muscle cells were found to be polarized.³⁹

On several occasions such contracting cells were removed from this position to the serum. In the serum they became spherical in shape and inactive. The same cells removed to the surface film stretch out to take a flattened spindle or irregular shape, grow and divide and look like sarcoma cells. Others were placed in the fibrin outside. They remained inactive or stretched out along the fibrin fibrilli and became inactive.³⁶

It was thus possible to show absolutely that function and growth in these heart muscle cells are determined by the environment and not by the cell. They are reversible phenomena. The change of a cell from the state of active function in the normal organism to the growth observed in cancer must be a normal reaction of the cell to a changing environment. Ribbert's contention had thus been realized.

While it was thus possible by means of the tissue culture to transform a functioning cell to a growing cell and vice versa, it has not been possible to transform a connective tissue cell into a muscle cell or an epithelial cell into any other type of cell. These cell types are determined by some stable internal mechanism in the cell. This fact one might have readily deduced from the study of cancer, however. There is no evidence that a cancer cell is other than the growing connective tissue or epithelial cell from which it arose. Teratomas develop only from egg cells. The egg cell can give rise only to multiple cell types. A

³⁹ Burrows, M. T. A Note on the Mechanism of Heart Muscle Contraction, *Am J Physiol* **45** 556, 1917.

growing smooth muscle or striated muscle cell resembles growing connective tissue cells

There is no evidence, as many recent authors have attempted to show, that the cancer cell is a specially differentiated cell, nor is there any evidence in the whole of embryology or experimental biology that such a cell may arise. On the other hand, it can be fully proved by the tissue culture that any cell may grow if given the proper surroundings and this growth may be independent. These surroundings are simply those which allow a certain high concentration of their archusia to accumulate about them. This archusia may be supplied from without or by the cells themselves when they are crowded into sufficiently narrow stagnant confines. Under the latter conditions, the growth of body cells becomes wholly independent. This is cancer. When supplied from other sources, their growth becomes dependent on the supply.

Function on the other hand comes into existence when the cell is so placed that the archusia remains in concentration only at one end. The type of function is determined by the more fundamental constitution of the cells in question. Connective tissue cells and epithelial cells stretched as the heart and striated muscle cells were stretched (described above), do not contract rhythmically. Smooth muscle cells so stretched contract rhythmically, but their contractions are not like the heart muscle and striated muscle cells, short and rapid, but are slow. They are exactly like the contraction peculiar to smooth muscle in the body.

In the body all functional cells have this arrangement. The nerve fibers are stretched between the dense brain tissue and an end organ. The gland cells are in contact with an active blood circulation on one side and a stagnant gland tube on the other. The archusia is washed away by the blood stream from their outer ends and accumulates at their inner ends. Functioning glands and nerves show electrical changes as muscle shows these changes³¹. While their function is different from that of the muscle cell, it is determined by the same organization and is evidently the result of an explosive breakdown of a polarized system. It differs from the muscle because of a different physical chemical make-up in the epithelial and other cells.

In the culture function had been forced on the heart muscle cells through a contraction of the clot away from the fragment. In the body the cells are forced to function through the development of intercellular tissue and blood vessels. In further proof for these deductions, it is noted that epithelial cells from glands of the body revert in the cultures to broad sheets of migrating and growing cells. Champy⁴⁰ found that if connective tissue is added to such culture the epithelial

40 Champy, C. La presence d'un tissue antagoniste maintient la differentiation d'un tissu cultive en dehors de l'organisme (Note preliminaire), *Comp rend Soc de biol* 76 30, 1914

cells are forced to form tubular structures again Drew ⁴¹ has confirmed these observations of Champy

CONDITIONS REGULATING GROWTH IN THE BODY

In the light of these facts, growth and function in the body take on quite different aspects. Function is something peculiar to an active circulation in properly arranged blood vessels. Growth is something peculiar to stagnant crowded cell areas where the circulation is poor. In this regard body cells are not different from any of the simple unicellular organisms. It is in the stagnant pool and not in the running stream that such life abounds. Wildiers had found that the growth of yeast depends on conditions identical with those outlined above for the growth of body cells. In 1923 experiments with a culture chamber which allowed the tissue to be washed continuously with a stream of serum were described. In these experiments it was found that washing prevents the growth of heart muscle cells but accelerates and makes their rhythmical contractions regular and forcible ⁴²

In the development of the animal body, it is interesting to note that growth is most active at the beginning. It wanes and is replaced by function as the blood vascular system develops. At maturity growth ceases except as it is necessary for repair, regeneration and in certain parts, such as, the nails, hair, sex glands and bone marrow.

Areas undergoing repair and various of the more permanently growing regions and cancerous tissues have been studied. In the wound, growth does not intervene alone at its edge but throughout the wide area of tissue behind its edge, which is suffering from the inflammatory slowing of the circulation. This growth behind the edge of the wound is proportional to the amount of congestion and slowing of the circulation. In the wound the greatest growth takes place in the stagnant wound areas where the blood vessels have been completely destroyed ⁴³

The distal end of the growing nail bed has a greatly reduced circulation. This area is supplied by a single layer of large sinusoids. These sinusoids are larger than the vessels which fill and empty them. The bone marrow has the same sluggish sinusoidal circulation. The growth in the sex gland is inside the tubules and follicles far removed from the blood vessels. Cancer is a densely cellular tissue supplied by irregular tortuous vessels few in number per unit cell area or by large dilated

41 Drew, A. H. Growth and Differentiation in Tissue Cultures, Brit. J. Exper. Path. **4** 46 (April) 1923

42 Burrows, M. T. Studies on Cancer, 4 papers, Proc. Soc. Exper. Biol. & Med. **21** 94, 1923

43 Burrows, M. T. Studies on Wound Healing. I, "First Intention" Healing of Open Wounds and the Nature of the Growth Stimulus in the Wound and Cancer, J. M. Res. **44** 615 (Sept.) 1924

sinusoids These vessels are being continuously destroyed in its more central parts Hemorrhage and necrosis are the result ⁴⁴

In the light of these facts it became evident, therefore, that cancer may be nothing more than the result of any substance or condition that can primarily build in the organism a dense mass of cells having a relatively poor blood supply Cancer is only such a tissue organization It is not a primary change in the cell

The proof for this deduction lay in showing that such an organization once induced can reproduce itself and destroy the organism and that the substance or conditions, such as coal tar, which induce it, act only to produce such an organization

In a previous article,⁴³ one of us has already discussed and given proof that such an organization once established preys on and destroys the surrounding less crowded and more vascular normal tissue All these normally arranged cells are attacked alike, including the endothelial cells of the blood vessels A mass of cells thus sufficiently crowded and stagnant not only reproduces itself through a growth of its cells but also the environment suitable for a continuation of this growth by its continuous destruction of its own vascular supply and that of the tissues about it This destruction of the blood vessels is greatest in the tumor The central parts of these tumors suffer necrosis The peripheral parts receiving sufficient oxygen continue to grow actively

This attaching of a less dense mass of cells by another denser mass was first noted in a study of the behavior of fragments of normal skin of embryos of various ages in the cultures In the early embryonic life, the epidermis is a single or a double layer of cells The underlying connective tissue or mesenchyme is a densely cellular layer As development proceeds in the embryo, the epidermis gradually becomes a thick cellular layer while the mesenchyme cells cease to multiply at an early time This layer expands rather by the laying down of intercellular substances, the connective tissue fibrils When this laying down of fibrils between the mesenchyme cells takes place, they become widely separated so that the number of cells per unit area in this layer becomes only a fraction of their original value When fragments of the skin of the younger embryos are cut from their circulation and placed in the cultures, the mesenchyme cells grow most actively and destroy and devour the protoplasm of the cells of the less cellular epithelial layer About fragments of older and older embryos, the activity of the mesenchyme wanes in the cultures and the epithelial layer becomes more and more active to dominate completely about the fragments of older

⁴⁴ Burrows (Footnotes 13, 42 and 43), Factors Regulating Cellular Growth and Their Importance in the Explanation of Cancer, *South M J* **17** 233 (April) 1924

embryos, as the mesenchyme had dominated in the earlier period. These active epithelial cells then destroy the cells in the less densely cellular connective tissue area. This growth of densely cellular layers can be greatly facilitated by adding embryonic extracts to the plasma.

Epithelial cells, as Fischer⁴⁵ has shown, do not grow readily in the cultures unless these embryonic extracts are added to the medium. Barta⁴⁶ has carefully studied the behavior of epithelial cells in fragments of the ureters of young rats. The ureters of rats are small. One millimeter fragments of the whole cross section can thus be placed in the culture without destroying the oxygen supply to all parts of this fragment. In a medium containing embryonic extracts, he found that the epithelial cells grew actively to invade the connective tissue and fat of the adventitia in a manner typical of true cancer. These growing cells reduced the muscle, blood vessels and connective tissue to a hyaline mass and destroyed the fat tissue. Their invading epithelial cells contained mitoses, irregular, large multinuclear cells, and were in every way identical with cancer.⁴⁰

Maximow has found the same phenomena in fragments of breast planted in a medium containing extracts of bone marrow.⁴⁷ Burrows had noted a sarcomatous overgrowth of the mesenchyme in embryonic fragments placed in pure plasma and the phenomena greatly exaggerated in a culture of these tissues into which an extract of embryos or cancer tissue is added. A few of these fragments transplanted into the subcutaneous tissue of an adult rat developed into true sarcoma.⁴⁸

Burrows has also found that this dominance of a more cellular and less vascular layer over a more vascular and less cellular layer occurs in wounds as well as in the tissue culture and in cancers. When the wound is open and suffering a congestion and slowing of its circulation, the granulation tissue grows actively in areas removed from the originally more densely cellular epidermis. As the epidermis moves in over the wound and the circulation is reestablished in the granulating area, growth ceases in this area, but continues in the more densely cellular epithelial layer until the granulations are reduced to a hyaline mass. The cells and blood vessels are destroyed by this growth of the epithelial layer, which continues until its blood supply is thus reduced and atrophy intervenes.⁴³

45 Fischer, Albert. A Three Months Old Strain of Epithelium, *J. Exper. Med.* **35** 367 (March) 1922.

46 Barta, E. Experimental Histological Studies, I, Some Factors Regulating the Morphology of Tissue (Ureter in Vitro), *Anat. Rec.* **29** 33, 1924.

47 Maximow, A. Tissue Culture of Mammary Gland, *Anat. Rec.* **27** 210, 1924.

48 Burrows, M. T. The Experimental Production of Malignant Ulcers in Rats, *J. Missouri State M. A.* **20** 145, 1923.

Such scars may become the site of cancers. It has also been found that the failure for the wound to become cancerous in every case is due to the fact that the epithelial layer under ordinary conditions does not become thick enough to overpower the resistance of the body outside. If this layer is stimulated to increase in thickness through the addition of growth stimulating substances to it, cancer may then intervene."

THE ACTION OF COAL TAR AND OTHER LIPOID SOLVENTS IN THE PRODUCTION OF CANCER

Before attempting an investigation of the action of coal tar and other lipid substances in the production of cancer, it seemed necessary first to study more carefully the general metabolism of fat by body cells. While many authors had studied the heat produced in fat metabolism and the various by-products into which fat may be changed, no analysis of the ingestion of fat and fat bodies by the cell had been undertaken.

In the early part of this article it has been shown that under the influence of a medium concentration of the archusia (S_2) the cell takes up fat, but does not digest it. Under the influence of higher concentrations of the archusia (S_3), the fat and proteins so taken up are digested and growth intervenes. How this fat is taken into the cell was then investigated by the senior author.

In this analysis it was noted that body cells, unlike many of the unicellular organisms, have no distinct mechanism for migration. They are simple fluids. They can migrate only into substances containing fats and proteins which have greater inertia than themselves. If the fats and proteins are in suspension and are mobile, they are drawn to the cells. If these same substances are fixed or large and have a greater inertia than the cell, the cells are drawn to them. The mechanism for migration in body cells is the mechanism for taking these necessary food substances into themselves. Migration is merely an adaptation of the growth reaction of these cells as function is an adaptation of the same reaction. These cells migrate or draw food into themselves by liberating a substance that has strong affinities for the cell as well as proteins and fat of the medium. This substance or substances has been named the *ergusia*, the laboring substance of the cell to distinguish it from the *archusia*, the driving substance of the cell.

In the plasma cultures the cells do not liberate the *ergusia* under all conditions, but only when the archusia accumulates to a certain concentration (S_2), as has been pointed out above. In the presence of this concentration of the archusia, the cell liberates the *ergusia*⁴⁹ which is adsorbed by the clot and fat in the clot. If the clot is held firmly through attachments to the glass surface on which it is placed, the cells are drawn out into it and the fragments spread and flatten out. If the

⁴⁹ Credit for the words archusia and ergusia is due to Prof. Thomas S. Duncan of the departments of Greek and Latin, Washington University, St. Louis.

clot is not attached, it is drawn in mass to the fragment. In the same manner, small, mobile fat droplets are drawn into the cell. Larger, less mobile masses of fats draw the cells to them.

In studying the *ergusia* in the different types of cells it is interesting to note that it differs in different cells. It has only one definite common property: it is adsorbed readily only by proteins and fats. The *ergusia* of the connective tissue coagulates blood to fibrin and serum. The *ergusia* of the epithelial cells has the same property, but these cells differ from the connective tissue cells in that they may later dissolve the fibrin formed. The *ergusia* of the leukocytes and lymphocytes does not form fibrin to any great extent. Their *ergusia* is readily adsorbed, however, by both the fibrinogen and fats.

In proof that the cells migrate and acquire their food by this means, it was not only found that their movements are often unassociated with any changes in their contour (they migrate without amoeboid movements) or evidence of other mechanical mechanisms, but also that they are exactly proportional to the liberation of this substance as such liberation is identified by the action of the *ergusia* of the connective tissue and epithelial cells on a plasma clot, and through the proof that the fat taken up by the cells becomes saturated with this *ergusia*.²³

In a short time after fats are eaten by animals their blood contains a large amount of this fat. It occurs in the blood in the form of suspended droplets. The plasma, prepared from such blood, is rich in fat. If a fragment of connective tissue is placed in a drop of this plasma, the cells become filled with these fat droplets. The fat moves rapidly out of the plasma into the cells. The fat has no coagulating effect on the clot. It prevents its coagulation rather than stimulates it. This fat after it is taken up by the cells acts differently. If the fat droplets are squeezed from these cells and placed in fresh plasma, they coagulate the plasma, stick to the fibrin fibrils thus formed and assume spindle shape quite the same as the connective tissue cells. When placed near other connective tissue cells, they repel these cells or are repelled and driven away from the cell exactly as one connective tissue cell repels another in the culture. These last phenomena continue, however, only until the *ergusia* which they have adsorbed is lost to the clot. Then they are adsorbed again by the cells when brought in contact with them.

In other experiments, it was found that this taking up of the *ergusia* follows the laws of adsorption or solution. There is a definite saturation point for *ergusia* in proteins and fats. These cells can migrate into these substances or draw them into themselves only until the concentration of the *ergusia* in the particles is balanced with that in the cell. Then all activity ceases.²⁶

In the light of these experiments, it became evident, therefore, that if one places in the tissue any viscid, more or less immobile and non-

digestible substance that has strong affinities for the ergusia it must draw the surrounding cells to it and away from their intercellular substances and blood vessels. It can thus disrupt the normal tissue organization up to the time it becomes saturated with this substance. This action accumulates the scattered cells of the tissue in dense stagnant masses about the absorbent and thus builds an organization in which the cells can accumulate their archusia and grow. It builds a nonvascular cancerous organization.

ACTION OF COAL TAR

Having established these facts, Jorstad⁵⁰ in this laboratory then undertook the study of the action of coal tar in the production of cancer. Jorstad injected small quantities, from 1 to a few cubic centimeters, of crude coal tar into the subcutaneous tissue of rats and also into embryonic tissue that had been cut into small fragments and injected under the skin of animals. These masses of coal tar were in some cases left for a long time and then removed. Others were removed at regular intervals of twenty-four hours and longer. In a few instances the coal tar was injected just beneath the epidermis so that the reaction of the epithelial cells could also be observed. In most instances it was injected into the subcutaneous tissue far removed from the epithelial layer.

The change induced by the drops of tar is a rapid migration of the cells of the tissue to their edges. In a few cases the tar broke up into a number of smaller drops. In most cases, however, it remained together as one large drop. These cells accumulating from wide areas in the tissue formed a collar around the drops of tar. The first cells to react to the drop underwent a granular degeneration in most of the experiments. The later cells remained intact. The cells chiefly attracted were the connective tissue cells and the endothelial cells of the capillaries. The capillaries themselves, therefore, disappeared through this movement and separation of their endothelial lining cells. The intercellular materials were little disturbed except that they showed a hyaline-like degeneration and became stripped of the fibroblasts, which normally lie scattered among them.

The action of the single drop of coal tar was limited in this capacity. Its action was most rapid at first. Then this activity ceased slowly, to stop in the course of a few days, as any substance that dissolves substances from a medium ceases as the distribution constant is satisfied between it and the medium.

In the sparsely cellular subcutaneous tissue, but few cells were thus collected by a single drop of coal tar. In the more cellular mesenchyme of embryos, the tar accumulated many more cells. This was also true

⁵⁰ Jorstad, L. H. A Study of the Behavior of Coal Tar on the Tissue, *Proc Soc Exper Biol & Med* **21** 67, 1923, The Behavior of Coal Tar in Embryonic and Adult Tissues, *J Cancer Res*, to be published.

when the tar was placed in contact with the more densely cellular epithelial layer. In the adult connective tissue, growth was never observed in the cells collected by the tar. In the more cellular mesenchyme of embryos in which a greater number of cells were collected, these masses became larger and more densely cellular. In these larger masses growth and division figures were seen. Against the epithelial layer of the skin the tar collects more cells, and in these denser masses of epithelial cells growth and division figures are always seen. This growth often leads to the formation of pearls and the picture of precancerous lesions. In the epithelial layer of embryonic tissues, typical cancerous lesions made their appearance.

The action of a single drop of coal tar is limited. It does not act to stimulate the cells to grow but only to draw them to it and accumulate them in dense masses about it. If these cell masses become sufficiently dense and large enough, growth may then later intervene as the archusia of these cells accumulates about them. To produce such large masses many applications of tar are generally necessary. With a single injection this is never accomplished in the animal under ordinary conditions. The cells collected soon suffer regression and hyaline changes, therefore. Eventually a hyaline scar surrounds the drops of tar. In a few instances after a time the drops of tar saturated with ergusia begin to migrate. They enter the veins and migrate to distant organs in the same manner as fat droplets squeezed from body cells migrate from fragments or other cells into the less saturated parts of the medium of the culture. The blood contains little or none of the ergusia peculiar to the fixed tissue cells. It acts as a solvent for this substance and attracts the tar.

These observations indicate, therefore, that the action of coal tar is not chemical, but purely physical. It acts merely by dissolving a lipid soluble substance of the cells. It is possible that in the fractional distillation of coal tar one will find a substance having a more efficient solvent action, but it is not likely that any important growth stimulating substances will be found, as many of the English authors have supposed. Any oil having a similar solvent action must act accordingly.

ACTION OF PARAFFIN AND PARAFFIN OILS

To prove the latter deductions more definitely, it became of interest to see how other oils act in this regard. Previous studies have shown that paraffin and paraffin oil applied frequently to a point on the skin will induce cancerous growths, as coal tar induces them. Mook and Wander⁵¹ noted that single injections of paraffin into the subcutaneous tissue of men lead to the production of tumors. They

⁵¹ Mook, W. H., and Wander, W. S. Camphor Oil Tumors, *Arch. Dermat. & Syph.* **1**: 304 (March) 1920.

found these tumors composed of drops of the oil enclosed in a hyaline, fibrous capsule. These tumors are similar, therefore, to the tumors that Jorstad found in animals injected with single drops of coal tar.

The paraffin in the cases studied by Mook and Wander had been injected into patients as a vehicle for camphor and other drugs. This discovery that this oil forms tumors led to the discontinuing of its use as a vehicle and the substitution of Mazola, or corn oil.

It is well known that corn oil has no immediate food value other than the fat it contains. It is largely free from vitamin. One of us (C. G. J.), in performing experiments with the Allen-Doisy hormone,⁵² noted that this oil also produces tumors. The Allen-Doisy hormone was dissolved in this oil and given hypodermically. It thus became of interest for us to study the action of pure corn oil when injected into the subcutaneous tissue. Previous authors had noted that many oils of the food when injected subcutaneously are not absorbed, but no one had studied the action of these oils on the tissues.⁵³

THE ACTION OF MAZOLA, OR CORN OIL

Thirty-nine rats, one monkey and one dog were used for these experiments. From 1 to 5 c.c. of the pure sterile corn oil was injected into the subcutaneous tissue of each animal. The oil was injected in each instance so that it formed one large oil droplet. This mass broke up quickly into numerous drops of various sizes. These drops remained unabsorbed. Each drop then became firmly encapsulated so that a multiple cystic tumor was formed, similar in each case to the one removed and photographed (Fig. 1). The nature of the changes that this oil induces in the tissue was studied by removing such tumors at regular intervals of one, two, three and one-half and seven days, after two and four weeks, and after two, three, five and seven months.

PROTOCOLS

EXPERIMENT 1—Two cubic centimeters of sterile Mazola oil was injected into the subcutaneous tissue of four rats. After twenty-four hours the oil tumors were removed. They consisted of a large number of small and larger cysts each enclosed in a grayish capsule, which varied from 1 to 3 mm. in thickness. One of these tumors was fixed in formaldehyd, the other three were fixed in Zenker's fluid, a part of the formaldehyd fixed tissue was cut with the freezing microtome and stained for fat. Other parts and the Zenker fixed tissue were dehydrated, embedded in paraffin, sectioned and stained with hematoxylin and

52 Allen, Edgar, Francis, B. F., Robertson, L. L., Colgate, C. E., Johnston, C. G., Doisy, E. A., Kountz, W. B. and Gibson, H. V. The Hormone of the Ovarian Follicle, Its Localization and Action in Test Animals, and Additional Points Bearing upon the Internal Secretion of the Ovary, *Am. J. Anat.* **34** 133 (Sept.) 1924, The Extraction and Some Properties of an Ovarian Hormone, *J. Biol. Chem.* **61** 711 (Oct.) 1924.

53 Henderson, Yandell, and Crofutt, E. F. Observations on the Fate of Oils Injected Subcutaneously, *Am. J. Physiol.* **14** 193, 1905.

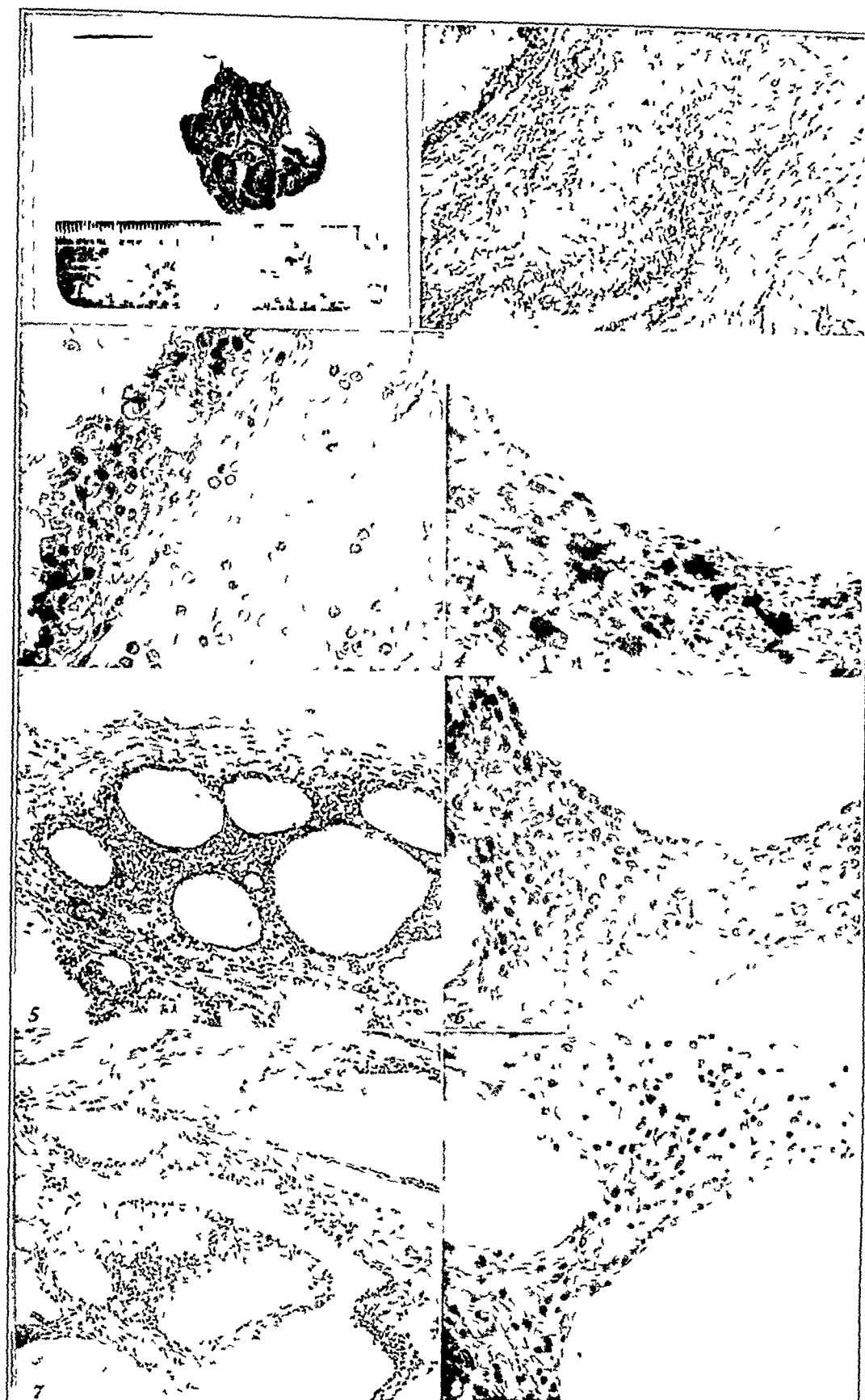


Fig 1—Carefully dissected 9 day old oil tumor from subcutaneous tissue of rat

Fig 2—Low power photomicrograph of section of oil tumor 24 hours old

Fig 3—High power photomicrograph of section of oil tumor shown in Figure 2

Fig 4—Photomicrograph of section of 24 hour old oil tumor that has been fixed in formaldehyd, cut with the freezing microtome, stained with sudan III and hematoxylin and cleared in glycerin. The oil has fallen out of the cavity. The small droplets are seen between the cells in the wall

Fig 5—Low power photomicrograph of section of 48 hour old oil tumor of rat

Fig 6—High power photomicrograph of section of oil tumor shown in Figure 5

Fig 7—Low power photomicrograph of section of 7 day old oil tumor of rat

Fig 8—High power photomicrograph of section of oil tumor shown in Figure 7

eosin At this early stage, one saw the oil carefully cut off from the surrounding fibrils of the fibrous tissue by a layer of cells varying from 1 to several cells in thickness The whole of the surrounding tissue was filled with cells evidently migrating toward the oil The immediately adjacent cellular capsule was ragged, the cells were not closely matted together in it A few had penetrated a short distance into the oil (Fig 2)

In studying more carefully the various parts of these sections, it was interesting to note that the extent of this cellular reaction varied in the different tissues into which the oil had been placed It was much greater about the drops placed in the more cellular fibrous tissue than in the fat tissue

The types of cells responding in most areas were chiefly large spherical or ovoid cells having a well formed vesicular nucleus Among these larger round cells in most areas one found also a scattering of small mononuclear cells, a few polymorphonuclear neutrophils and a larger number of polymorphonuclear eosinophil cells (Fig 3) The relative number of the latter cells also varied with the regions into which the drops were placed Among the more numerous blood vessels of the subcutaneous tissue, one saw a larger proportional number of lymphocytes and leukocytes than in the less vascular fibrous tissue and fat

With this movement of cells toward these drops of fat, tiny droplets of fat were continuously breaking off from the larger one and moving out into the tissue This moving out of small fat drops was in proportion to the movement of the cells toward the larger drops These small fat droplets could be readily brought into the field of vision at all periods of the development of these tumors by staining the frozen formaldehyd fixed sections with sudan III and hematoxylin and clearing the section with glycerin (Fig 4)

At first we thought that these large mononuclear cells with a vesicular nucleus were wandering cells of some sort or another, as many other authors have considered them It soon became evident, however, that this was not true As seen in the section of the tumor taken at a later period, these cells were fibroblasts that had loosened from their attachment to their intercellular fibrils and endothelial cells, which had separated themselves from the capillaries through the action of the oil on them

By means of the tissue culture, it was possible to determine fully that the shape of the fibroblasts is not determined by any internal organization peculiar to them, but to chemical and physical reactions that they induce in the environment about themselves They are simple fluid systems When connective tissue cells are placed in body fluids containing fibrinogen, they coagulate the fibrinogen to fibrin and stick to the side of the fibrin fibrils They are fastened to the fibrin by the *ergusia* These fibrin fibrils, as Hertzler²⁵ has shown, become the intercellular fibrils of the connective tissue The oil dissolving this *ergusia* not only loosens these cells from their attachment to the fibrils, causing them to round off as any drop of fluid rounds off under these conditions, but it also draws them to it At later periods after the oil droplets have become saturated with the *ergusia*, the cells slowly assume other shapes according to the shape of the proteins which they coagulate in their environment²³

EXPERIMENT 2—Four rats were injected the same as in Experiment 1, and the tissue removed was studied and treated in the same manner The difference between this experiment and the first one was that the tumors were removed after forty-eight hours At this period the cells had ceased to migrate to any extent from the tissue outside The cells at the edge of the oil droplets

had become more closely packed together into a layer, several cells in thickness (Fig 5) The inner layer of cells had in many places formed a continuous layer of cuboidal or flattened cells much like endothelial cells Outside this inner cuboidal layer, the large mononuclears were stretching out more or less in many places to spindle and irregular shaped cells These changes were suffered only by the large round cells with vesicular nuclei The lymphocytes and leukocytes remained scattered among them as before (Fig 6)

EXPERIMENT 3—In this experiment one rat was injected in the same manner as the others The tumor was removed after three and one-half days It showed no important differences in its morphology from those removed after forty-eight hours

EXPERIMENT 4—Four rats were injected as in the other experiments The tumors were removed after seven days At this period the picture had changed little from that of the earlier periods, except for a further organization of the cells about the fragment into a definite connective tissue (Fig 7) Most of the large mononuclear cells were stretching out to take a spindle shape along the forming intercellular fibrils, or had alined themselves into a layer of flattened or cuboidal cells about the oil droplets (Fig 8) The fine intercellular fibrils noted in the specimen removed at forty-eight hours (Fig 6) had now become more prominent, a true intercellular fibrous tissue had made its appearance in many places (Fig 9) Most of the polymorphonuclear and small lymphocytes still remained scattered among these cells They had taken no part apparently in the actual formation of the capsule, however

EXPERIMENT 5—Twenty-two rats were injected as in the other experiments The tumors were removed from these rats under ether at fourteen days, one month, two, three and seven months The tumors were fixed either in formaldehyd or Zenker's fluid and stained with hematoxylin and eosin or hematoxylin and sudan III In most of the specimens the changes were a gradual increase in the organization and then a hyalinization of the capsule about the oil droplets The oil remained unchanged during this later period (Figs 10, 11 and 12)

While the oil remained unchanged in practically all these cases, in one rat it was apparently absorbed This absorption took place slowly, and as the oil disappeared the circumference of the cellular wall either shortened or the tumor collapsed It was interesting to notice that the cells in the capsule of such cases of absorption did not tend to lay down intercellular substances but remained closely packed together much the same as they were at the beginning They also showed some evidence of growth in that their nuclei contained more chromatin and their cytoplasm stained more deeply (Fig 13) Mitosis also was observed in one of these cases There was never any evidence of the oil stimulating the cells to grow in any of the other cases The oil attracted the surrounding cells of the tissue to it, but it did not excite growth in these cells It acted rather to cause them to degenerate Evidences of growth were seen in these sections only when the oil was placed in a sufficiently cellular tissue so that a large number of cells became massed at its periphery When the number of cells became large enough, as in Figure 15, then one saw sharply staining cells Growth in these cases is evidently a secondary phenomenon and the result of the massing of the cells together It was secondary to the original action of the oil which pulled them into this mass

EXPERIMENT 6—One dog was injected in the same manner as the rats The tumor was removed after three weeks It was interesting that in this animal the oil was being slowly adsorbed as in the case of the one rat described above (Fig 14) The cells about the absorbed oil droplet showed evidences of stimulation The conditions leading to growth and the absorption of the oil in these cases were not determined

EXPERIMENT 7—One monkey was injected with 5 cc of Mazola oil The tumor was fixed in formaldehyd after nine days The oil had broken up into numerous droplets Each of these tumors was enclosed in a cellular fibrous

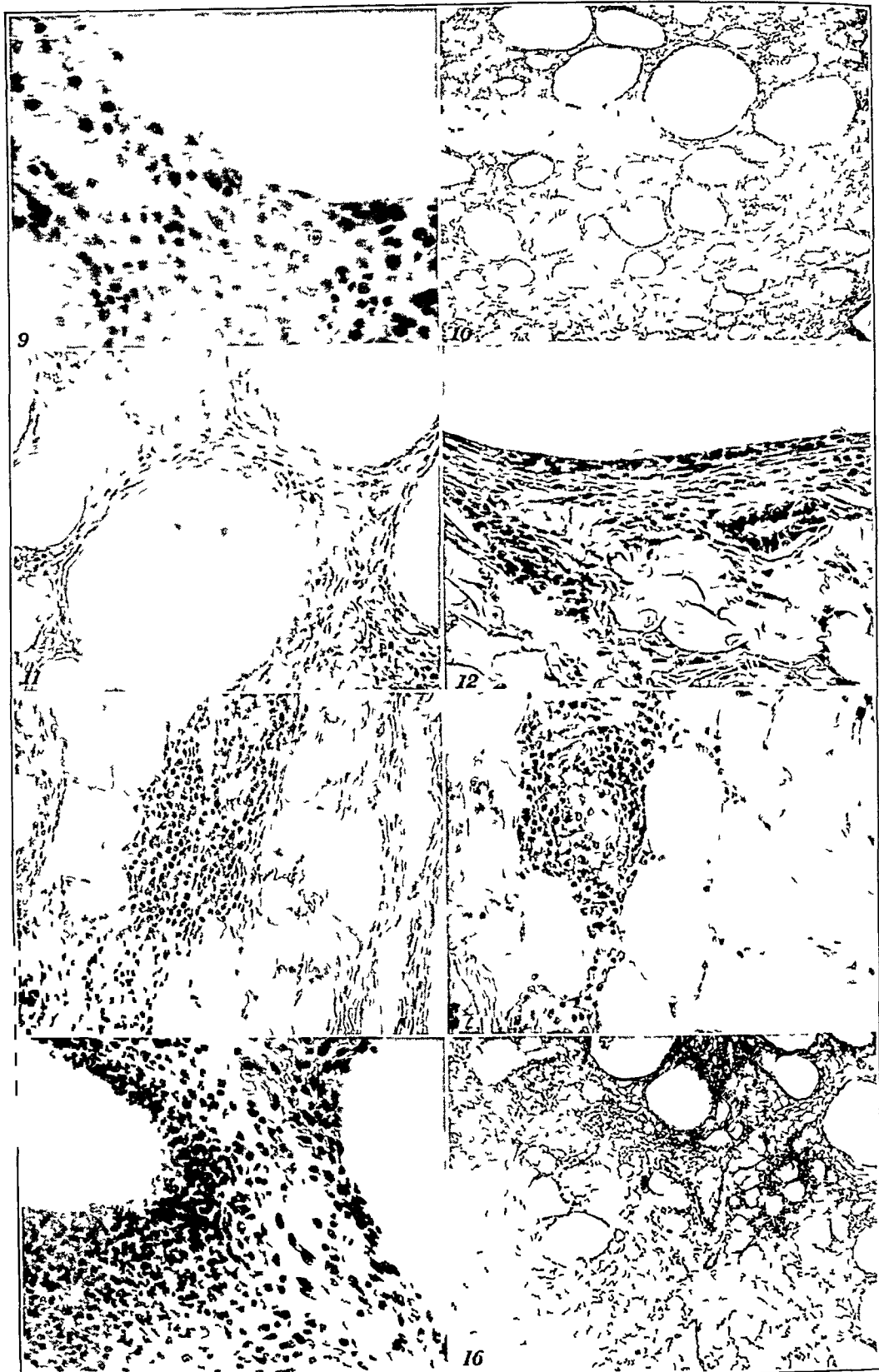


Fig 9—High power photomicrograph of another part of section of oil tumor shown in Figures 7 and 8

Fig 10—Low power photomicrograph of section of 22 day old oil tumor of rat

Fig 11—Low power photomicrograph of section of 7 months old oil tumor of rat

Fig 12—High power photomicrograph of section of 24 day old oil tumor of rat

Fig 13—Mass of cells marking site of oil cyst in which the oil was absorbed

Fig 14—Section of wall of oil tumor from subcutaneous tissue of dog, the oil has been absorbed and the circumference of the tumor has reduced accordingly

Fig 15—Dense mass of cells at edge of oil droplet showing evidence of growth in 7 day old oil tumor of rat

Fig 16—Low power photomicrograph of section of 9 day old oil tumor from subcutaneous tissue of monkey

capsule like that seen in the majority of the rats. None of the oil had been absorbed (Fig 16)

EXPERIMENT 8—Two rats were injected with pure Mazola oil that had not been sterilized. The tumors were removed after seven days. The picture in these cases was different. The oil was surrounded by a mass of fibrinous exudate filled with polymorphonuclear cells. This typical inflammatory reaction was quite different from the picture of the simpler migration of wandering and fixed tissue cells to the sides of the sterile oil droplets.

Mook and Wander noted giant cells in the hyaline capsule surrounding droplets of paraffin oil. They considered that the oil acted as a foreign body. They called these giant cells foreign body giant cells. The tumors they classified as sarcomas.

It must be pointed out that paraffin oil applied frequently to a given place in the skin will lead to cancerous production. What Mook and Wander studied were single injections. The oil that had been injected in many of their cases was not pure paraffin oil, but oil containing camphor. This substance excited a true inflammation. There was an exudation of fluid and fibrinogen from the blood vessels, and when necrosis intervened polymorphonuclear cells made their appearance.⁵⁴ The fibrinogen and fluid formed a thick mass about the irritant or diluted it. The fibrinogen clotted to a gel. As stated above, oxygen diffused readily into such clots no more than from 0.5 to 0.7 mm. The cells become abnormal beyond this distance from the source of oxygen. Barta⁵⁵ has recently studied these abnormalities and finds that cells situated a distance greater than 0.7 mm from the air surface of the layer of plasma medium of a culture change into reticular, epithelioid and giant cells. These cells are not in any way concerned with foreign bodies but owe their formation entirely to a reduction in their oxygen supply. Fibroblasts, certain wandering cells and other tissue cells will show these changes. The giant cells noted by Mook and Wander can only be indicative of such an inflammatory process. This same inflammatory process we noted about drops of unsterile corn oil. The sterilized oil acts only to dissolve the ergasia of the cells. Since the ergasia has strong affinities for the oil as well as the cells, the oil is thus drawn toward the cells and the cells to the oil. The larger oil droplets become encapsulated by drawing the cells to them from the tissue and blood vessels in proportion to the original number of these cells in these localities. Smaller droplets of fat are seen among the cells. They are drawn from the larger drops by the same process that the cells are drawn to these larger drops.

54 Burrows, M. T. Neuritis of the Cranial Nerves in Lethargic Encephalitis and the Differential Anatomic Diagnosis Between It and Acute Polio-myelitis, *Arch Int Med* **36** 477 (Oct.) 1920.

55 Barta, E. Experimental Histological Studies II, Giant Cell Formation and Fat Metabolism in Relation to Oxidation (Lymph Nodes in Vitro), to be published.

While many previous authors have looked to the discovery of some hormone or growth stimulating substance in coal tar and other lipoids capable by long use of inducing cancer, others have appreciated that such is probably not the case. They had noted that the oil acts to cause the cell to degenerate rather than grow (Bullock and Rohdenberg⁹ and Champy¹⁰). What stimulated the eventual proliferation of these cells they could not determine. The tissue culture has answered this question. It is the result of the crowding of cells induced by the oil attracting the cells of the tissue to it away from their intercellular substances and blood vessels.

It is now well established that to induce cancer with coal tar and other lipid solvents these substances must be applied frequently over a long period of time. The reason for this is clearly shown above. A single drop of oil becomes quickly saturated with the ergusia, or lipid substance, of the cell. It can pull only a few cells to it. With each addition of fresh oil to the same place, more cells are pulled in until finally there is built a stagnant mass of cells of sufficient size to overcome the growth energy of the surrounding tissue.

COMMENT

Previous work on cancer has shown that it may be induced by a variety of conditions and substances. It follows on the site of chronic inflammation, it may arise in many benign congenital and other types of benign tumors and congenital defects, it may be induced by coal tar, other lipid solvents, roentgen rays, radium, bacteria and certain animal parasites. While many of these substances and conditions were shown capable of inducing cancer, it was also noticed that they are unimportant in the eventual course of this disease. Cancer is an independent growth of body cells which may be induced by any one of these conditions and substances but, once established, then proceeds independently of them. The first important problem to be solved in cancer is the conditions allowing such an independent growth of the cells.

This problem was investigated by one of us (M. T. B.) by means of the tissue culture. It was found that single cells cannot grow in a simple body medium, such as blood plasma. Growth can intervene in this medium only about densely cellular fragments and it proceeds about these fragments only when they are placed in a very small stagnant quantity of this medium, which is well supplied with oxygen. This growth depends on the accumulation and maintenance of a certain concentration of a primary product of the cells about them. This product is formed by them through the oxidation of food materials. It is soluble in the blood, in serum and in isotonic sodium chlorid.

solution This substance or substances has been called the archusia, or the laboring substance, of the cell

The body cell is a drop of fluid cytoplasm in which floats a fluid nucleus It also contains fat droplets, centrosomes, mitochondria and other formed bodies floating in its cytoplasm These cells have no organization other than centrosomes (active only in cell division) for transforming their energy into work These cells can induce reactions between certain food substances and oxygen with the formation of the archusia The archusia acts on the cell in proportion to its concentration In low concentrations (S_1) it has no effect In a medium concentration (S_2) it acts to liberate another substance from the cell's protoplasm, the ergusia The ergusia is a lipid soluble substance readily adsorbed by fats and proteins Small mobile particles of proteins and fats are drawn into the cell as this substance is liberated into a medium in which it can diffuse Larger masses of proteins and fats draw the cells to them The cell grows by this ability to take up these proteins and fats Its migration is the result of the same reaction and occurs when the cell meets masses of proteins and fats having a greater inertia than itself The ergusia is not the same in the different cells It is highly selective in its action on proteins but not on fats All cells are attracted by fats The ergusia of the connective tissue cells coagulate fibrinogen to fibrin while the ergusia of the leukocytes and lymphocytes does not The different cells in the body differ from each other in their chemical make-up They grow always to reproduce themselves This specificity in their growth may be readily referred to the specificity of their ergusia and the specific selection of food substances by it

In high concentrations the archusia (S_3) causes the proteins and fats to be digested, the cell grows and divides by mitosis In all higher concentrations (S_4) the cell is digested and destroyed

The archusia is apparently the same in all cells The archusia extracted from one cell sets up the reactions mentioned above in other cells None of these cells except possibly the striated adult muscle fibers can retain their archusia For them to grow independently they must be crowded, therefore, into a small stagnant area in which their archusia cannot escape from about them Since the archusia is not specific, these same cells can be made to grow dependently, however, by supplying archusia from the outside In such cases their growth must be limited always to the supply, and wholly under the control of the outside forces supplying this substance

In the light of these facts, it became evident that cancer may be none other than the result of a primary reorganization of tissue in the body There was no evidence that the independently growing cells of the culture had changed primarily The change leading to their growth

was in the environment. Applying these principles to the study of growth as it is observed in the body, it became evident that all growing tissues of the normal organism are cellular and have a stagnant circulation and that this stagnation and cell crowding reaches its greatest development in cancer. The question therefore arose, Is cancer other than the result of the building of a dense mass of cells free from blood vessels? The proof of this deduction lay in showing that such a mass once built will reproduce itself and that the substances and conditions that lead to cancer act only to build such a tissue organization.

It was found by means of the culture and a study of wounds that any dense, stagnant mass of tissue may not only grow but also destroy readily any less dense and stagnant tissue. Not only the surrounding tissue cells but also blood vessels suffer in this destruction. Such a mass once established can thus continuously reproduce itself through a growth of its cells and the continuous destruction of its blood supply.

The action of coal tar and corn oil in the production of cancer have been investigated. It has been found that they have no stimulating action on the growth of the cells, but act only to disrupt the normal organization of the tissue and build a densely cellular tissue relatively or wholly free from blood vessels and intercellular substances. These oils act to collect these cells merely by dissolving the ergusia, the lipid-like substance of the cell. The cells are drawn to the edge of the drop merely through this adsorption by the oil of their ergusia. The ergusia liberates the energy for the migration of the cell, through its strong affinities not only for the cells but also for the oil. It thus decreases the surface tension of the cell in the presence of the oil.

A single drop of coal tar, corn oil or paraffin is limited in this capacity. It soon becomes saturated with the ergusia. It cannot draw a sufficient number of cells or form a sufficiently large mass of these cells for them to overcome the resistance of the outside and thus take food to themselves. With each new addition of the oil, more and more cells are drawn to the drop until a cancer is built.

We have not studied the action of roentgen rays, radium and animal parasites in the laboratory. It must be pointed out here, however, that the only tapeworm larva known to induce cancer is the cysticercus of the cat tapeworm. This larva produces changes in the tissues of the host which are different from those produced by the larvae of tapeworms, which do not induce cancer. It induces a densely cellular tissue capsule about it. This capsule is a dense mass of fibroblasts, while that induced by the echinococcus, a noncancerous producing parasite, is a hyaline, fibrous one containing very few cells. Wolbach,⁵⁶ in 1909, had shown that the roentgen ray acts on the tissue to reduce

⁵⁶ Wolbach, S. B. The Pathological Histology of Chronic X-Ray Dermatitis and Early X-Ray Carcinoma, J. M. Res. **21** 415, 1909.

the blood vessels and change them. It acts, therefore, also to build a cancerous organization.

Bacteria are now being studied carefully. They act differently from the tar to produce, however, the same nonvascular cellular organization in the tissues. Smith had noticed that *Bacillus tumefaciens* when introduced into the tissues of a plant induces the cells to proliferate actively. In studying the action of this organism in the animal, we find it causes the cells with which it comes into contact to proliferate without the formation of normal body structures, such as the blood vessels and intercellular substances. It acts to produce by proliferation a local dense mass of cells that is identical to the masses formed by the drops of oils through collecting the scattered cells of the tissues about them. Extracts of embryo and Berkefeld filtrates of malignant tumors, which are rich in archusia, act in the same manner to stimulate the tissue cells to proliferate. Drops of oil act to disrupt the normal organization of the tissue and build one peculiar for cancer in that they are able to attract the cells from the tissue to them (Fig. 17). The bacteria produce the same organization by stimulating the cells that lie between the blood vessels to proliferate and thus to form a crowded cellular mass (Figs. 18 and 19). The blood vessels do not grow to any extent because the stimulus is continuously washed away by them and cannot concentrate on their lining endothelial cells.

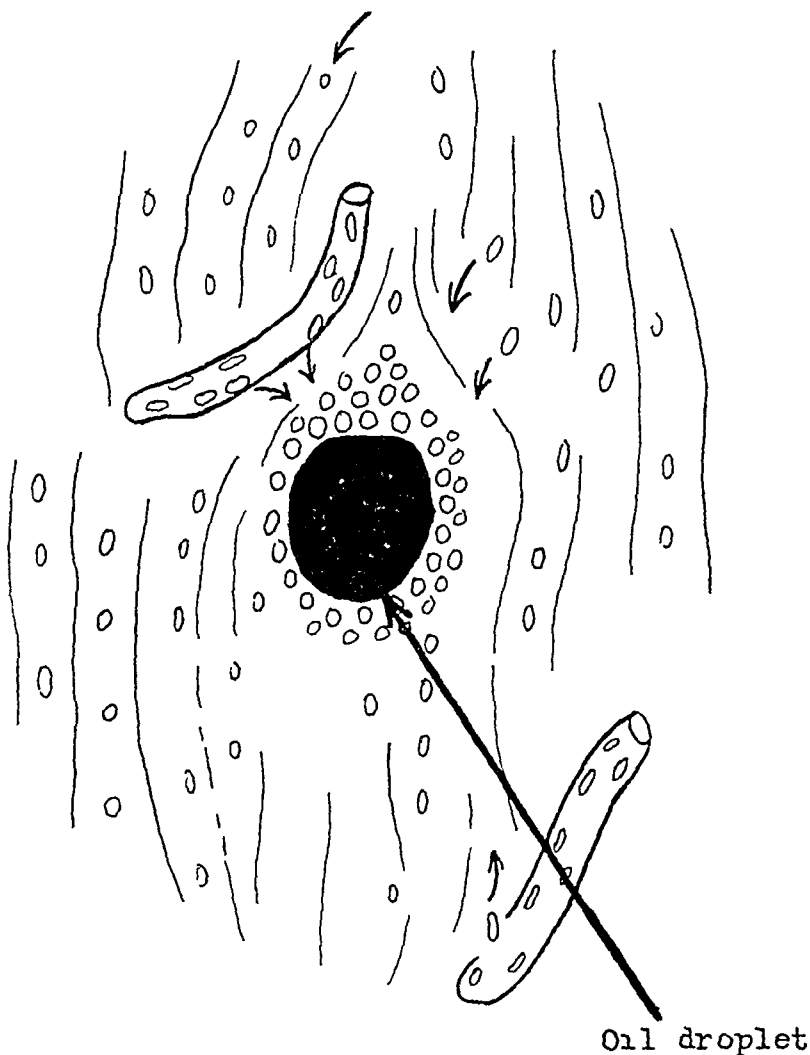
The latter experiments on the action of bacteria are interesting in that they show that certain bacteria can liberate growth stimulating substances not unlike those liberated by body cells.

In the further study of the properties of tissue cells, it has been shown that these simple active fluid systems when polarized or placed in an environment in which the archusia can concentrate only at one of their ends, suffer a rhythmic explosive breakdown of either the eigusia liberated at the one end where the archusia is high or the substance adsorbing it in the environment. These breakdowns are expressed in muscle by contraction, in the gland cells by secretion, in the nerves by conduction. Function is the result of such a polarization. In the body this polarization is induced by the interspersing of blood vessels between double columns of cells. In the culture function is produced by placing the same conditions about the cells. The blood vessels bathing one end of the cell remove the archusia formed at this end of the cell. It accumulates at the other end of the cell away from the blood stream.

In the light of the foregoing observations cancer maintains when the cells are crowded together in an area having a greatly reduced blood supply. Function is a property of a rich blood supply in properly arranged blood vessels. It is something which takes place at the expense of the growth reaction of the cells. It reaches its greatest development in an environment that must inhibit growth completely.

unless the cells are supplied with archusia by other means. Function is something which has been imposed on the cell by outside forces and not something which the cells will tend to develop at their own initiative. The question confronted us, What is the nature of these outside forces?

As has been noted, single cells isolated in a drop of plasma and groups of cells washed with a stream of serum cannot grow. The same



Oil droplet

Fig 17—Manner in which a drop of oil attracts cells when placed in the tissue, it disrupts the normal organization of the tissue and builds a cancerous organization

cells can be made to grow by adding archusia to these mediums. As stated above, such growth is not independent but dependent on the source of the archusia. In the animal organism there is no evidence to show that the cells lose their power to grow at any time. The prevention of an active, independent growth of these cells and function in the normal organism is wholly dependent on the organization of the normal organism, the arrangement of its blood vessels and cells

In other studies in this laboratory, it has been found that the cells of the normal body do not grow independently at any time from the egg to the death of the adult. At no place are they crowded sufficiently and their environment sufficiently stagnant to allow them to grow without an outside source of archusia. In cancer conditions are different, the organization about the cells has changed to one in which the cells are crowded sufficiently and their relative circulation sufficiently reduced for them to form enough archusia and retain it in a concentration ample for them to grow independent of any outside source.

In the light of these facts it becomes evident, therefore, that growth in the body must be at all times dependent on a source of stimulus from

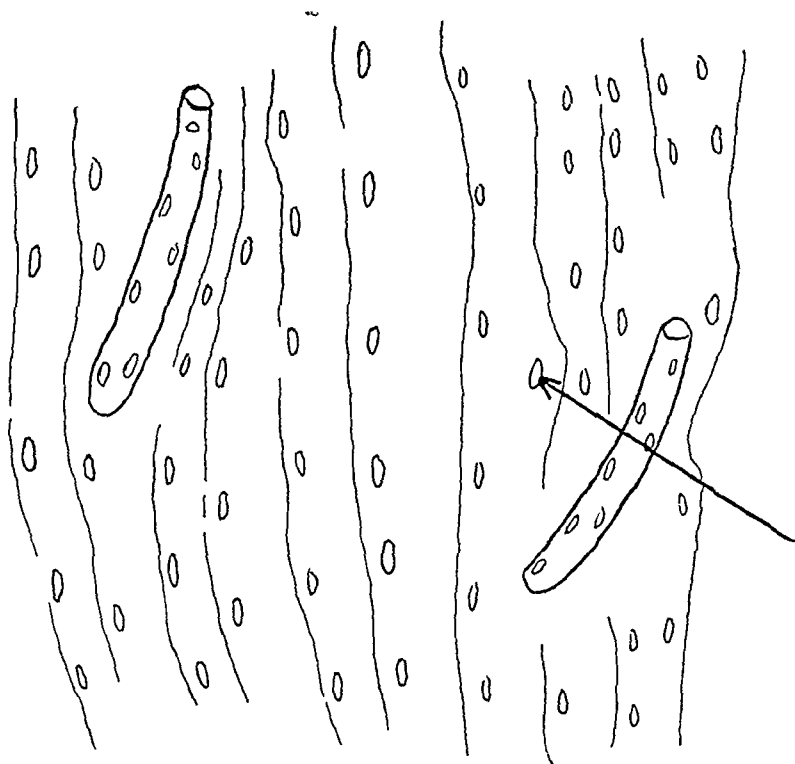


Fig 18—Site of introduction of tumor forming micro-organism or extract of an actively growing tissue

the outside—the mother, the yolk of the egg or the food. It is a well known fact that one cannot exist on proteins, fats and carbohydrates alone. Other accessory food substances are necessary. These have been termed vitamins. It has been shown above that when *B. tumefaciens* is introduced into the tissues it stimulates the cells to proliferate. They grow to form a densely cellular nonvascular mass. The same is true for extracts of embryos or any other actively growing tissue. Under normal conditions these substances cannot gain entrance to the body except through the food. In the food they must enter the blood vessels first and then gain access to the cells. Under such conditions they will not act to build a cancerous organization. The blood vessels must

suffer the greatest stimulation. They must grow, therefore, in excess of the cell between them and form a vascular rather than a nonvascular tissue.

In the light of these facts, it became of interest for us to study the action of *B. tumefaciens* and the extracts of embryos when injected and when fed to the animal. One of us had already noted that extracts of actively growing embryos when injected into the skin stimulate an

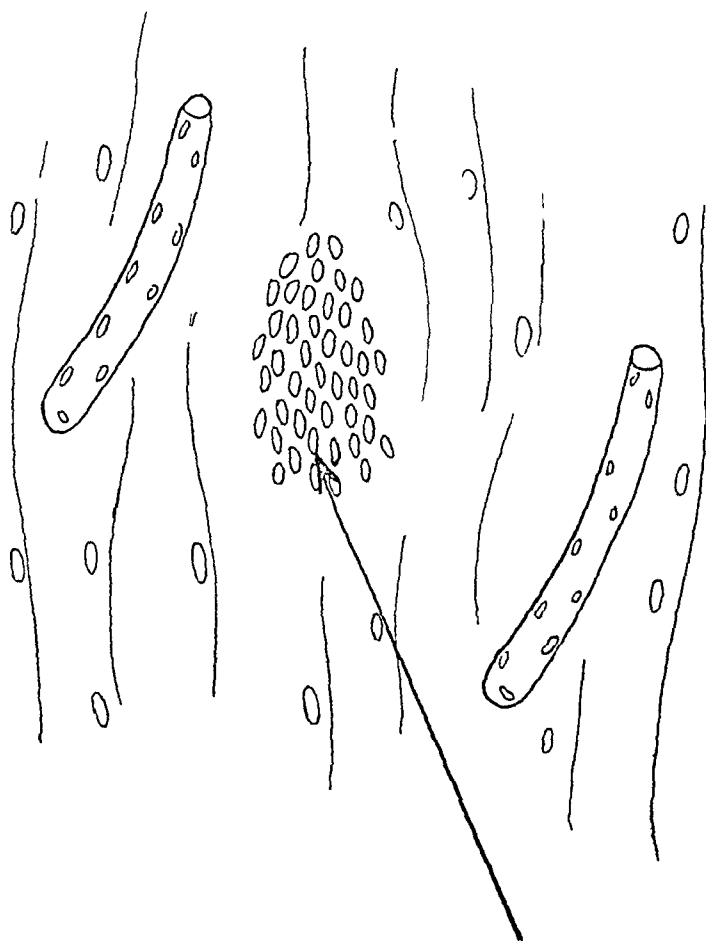


Fig 19—Result of introduction of tumor forming micro-organism or extract of an actively growing tissue into the tissues. The cell proliferates without the formation of intercellular substances and blood vessels. These bacteria or tissue extracts by stimulating the cells to proliferate accomplish the same result as oil in attracting the scattered cells of the tissue.

active growth of cells and cancers.⁴⁸ We are now investigating the effect on the growth of young rats of feeding *B. tumefaciens* and extracts of embryos.⁵⁷ Two day old and 5 day old cultures of *B. tumefaciens* (Smith) were fed to rats in a diet free from vitamin B and in a diet free from vitamin A. The rats fed on 2 day old cultures in a

⁵⁷ Burrows, M. T. Studies to Determine the Biological Significance of the Vitamins, *Proc Soc Exper Biol & Med* 22:241, 1925.

diet containing vitamin A (butter) grew as well as those fed on the same diet containing autolyzed yeast instead of the bacillus. The rats fed on the 5 day old culture grew as actively. Extracts of embryos that had been shown to be rich in the archusia have also been fed. These extracts act only as vitamin B substitute. In other experiments we have sought for stimulating substances in the glands of internal secretion. The studies on the ovary have now been completed. The Allen-Doisy hormone extracted from the follicular fluid of the ovary is found to be rich in an active growth stimulus. Whether this stimulus acts as substitute for the vitamins we have not determined.

From these observations on the effect of feeding these substances on the growth of the animal, it becomes evident, therefore, that the control of body growth, its form and function must depend on three factors: (1) a complex egg cell capable of giving rise by division to multiple cell types, (2) an absence of the power of the cells of the body to retain their archusia, and (3) an active supply of archusia from an external source aided probably by certain glands of internal secretion.

It has been possible for us to show in the laboratory that when a stimulus is introduced into the tissue it stimulates an active growth of cells and a cancerous organization. When fed it produces a normal vascular tissue. When placed in the tissue it stimulates the cells to grow without organization. From the food it enters the blood vessels first, and stimulates them to grow more than the intervening tissue cells. A vascular-functioning tissue is thus formed.

The bodies of higher animals must be, therefore, the result of an evolutionary development. Their function and normal growth is not a property peculiar to their cells, except in an environment rich in growth stimulating substance, the archusia or something similar. This substance forcing its way in from the outside builds the vascular system. Function is not a normal act of the cells, but something imposed on them by the body organization thus controlled from without. The bodies of higher animals survive, grow and function only through this outside aid. Such stimulants are formed probably only by living cells. The body exists through preying chiefly on lower nonfunctioning growing forms. While the glands of internal secretion must aid in the directing of these stimuli, the chief source of these stimuli is probably from without.

In the cancerous state the cell alone reaches its full independence. In further proof for this deduction, it is interesting to note that Cramer⁵⁸ has found that the growth of transplanted tumors of rats is not influenced by the absence or presence of vitamins. We have

58 Cramer, W. Dietary Deficiencies and the Growth of Cancer, Eighth Scientific Rep. Imper. Cancer Res. Fund, London, p. 17.

repeated and confirmed these experiments⁵⁹ In cancer the cells are able to live an existence independent of specific substances in the diet In the crowded, stagnant environment of cancer they can conserve their energy, the archusia, and build their parts from simpler substances

The cancer cell is not primarily different from the normal cell The primary change in cancer is a reorganization of the tissue so as to produce a densely cellular mass having a relatively poor circulation While it has been shown from the work of Nuzum,¹⁶ Blumenthal, Auler and Meyer¹⁵ that a certain number of cancers of man undoubtedly owe their origin to bacteria, it must be clearly emphasized here that this does not indicate that cancer is infectious Cancer may be produced by any one of a number of conditions and substances that primarily build a dense mass of cells in the organism in such a manner that the circulation is so reduced that this mass receives at its periphery an ample oxygen supply, but is otherwise stagnant The conclusions drawn by Oxner⁶⁰ and Nuzum¹⁶ are not based on the facts at hand and are thus wholly uncalled for and may do harm⁶¹ Blumenthal, Auler and Meyer have carried their work further than Nuzum They found it impossible to draw such conclusions⁶² Cancer is the normal means of the cell to survive when unaided from without It must always be the normal outcome of a certain number of aging organisms

The difference between the cancerous tissue and the normal is the difference between the greater conservation of growth energy in the cancer than in the body outside Our immediate hope for the medical treatment of cancer does not lie in an ability to equalize the energy in the body over that in the cancer, but in finding some means to overwhelm the growing cancer cells If we raise the amount of archusia in the blood to that in the cancer, the growth of the cancer must cease, but function cannot maintain under these conditions Function depends on the absence of, or a very low, archusia at the end of the cell in contact with the blood stream and a high value at the opposite end The amount of function is the difference in these energy values When we demand increased function, the circulation increases When we eat a big dinner and load our blood with growth stimulus from without, we become

59 Burrows, M T, and Jorstad, L H Unpublished notes

60 Oxner, A J Cancer Infection, Surg, Gynec & Obst **40** 336, 1925

61 Soper, S A The Application of Facts and Opinions Resulting from Laboratory Experiments to the Practical Work of Cancer Control, Surg, Gynec & Obst **40** 334, 1925

62 The same evidently applies to the recent work of Gye and Barnard reported in the lay press and in J A M A **85** 368 (Aug 1) 1925, at the time this proof was being corrected The Rous chicken sarcoma has long been suspected as being due to a bacterium This is not true of many of the sarcomas and carcinomas of rats and other animals Gye and Barnard will undoubtedly find that extracts of any actively growing tissue, many bacteria and other substances capable of stimulating cells will induce cancer if properly introduced (Footnote 48)

dull and drowsy After a day of active blood circulation, we become exhausted, our blood pressure drops, we sleep The cells must be allowed to grow, to recuperate their loss of substance through function

We have found, however, that when the archusia is raised in the medium of a tissue culture to a certain high concentration (S_4) the cells are digested Cancer cells and actively growing embryonic cells can be made to suffer such digestion by adding to the medium containing them only one-tenth of the amount of archusia capable of stimulating growth in fresh normal adult tissues Whether this can be used for treatment is yet to be investigated It is possible that other more simple means will be found to destroy the cancer without destroying normal cell The means to discover such substances or condition will probably be found in a more careful analysis of roentgen rays and other substances inducing cancer and in more study of the details of cellular growth and function

CONCLUSIONS

1 The tissue culture has given us an entirely new idea of the structure and activity of body cells Our earlier ideas had been gleaned entirely from a study of complexly organized unicellular organisms and a study of the morphology of body cells The tissue culture has allowed us to study for the first time the mechanism of growth and function in these cellular elements They are one of the few cells in nature which have a simplicity of structure sufficient to make such an analysis

2 Cancer is an independent growth of these cells It may be induced by any one of a number of conditions and substances Once established, it proceeds independently of these causative agents

3 An active independent growth of body cells depends on a crowding of the cells together in a stagnant area or an area having a relatively poor blood supply Cancer has this kind of an organization

4 It has been found that such an organization can reproduce itself through the fact that it destroys through its growth the normal tissue and blood vessels about it

5 The action of drops of corn oil and coal tar on the tissue has been investigated It has been found that they act to build a dense, stagnant mass of cells by drawing the tissue cells to them and away from their intercellular substances and blood vessels

6 The bacteria that induce cancer act in the same manner to build a densely cellular stagnant mass of cells, but by a different means They induce such a mass of cells to form by stimulating the cells of the tissue to proliferate without forming to any extent intercellular substances and blood vessels

7 These factors of stagnation and cell crowding become important for growth because the growth of body cells depends on a certain con-

centration of a primary product of the oxidation of the cells, the archusia. This substance is soluble in blood. The cells cannot retain it in quantity. Its concentration is at all times under the control of the environment.

8 Function is likewise determined by the environment. It maintains in an environment rich in blood vessels so placed that they pass close to one end of the cell. The other end of the cell away from these vessels accumulates archusia and is active. The end near the vessels is inactive. Function is the result of an explosive breakdown of such a polarized cell. This was proved by a direct analysis of isolated functioning cells in the cultures.

9 Body cells can be made to grow not only through the accumulation of their own archusia, but by adding archusia from outside sources.

10 Function is something that maintains only in an environment that inhibits an independent growth. It occurs only in a richly vascularized tissue. Function is something imposed on the cells of the body. For them to survive in such a state they must be supplied with growth energy from other sources. This functioning tissue is formed and maintained by growth stimuli entering the blood vessels from the digestive tract. These stimuli act in the capacity of vitamins. They represent the growth energy of other living things.

11 Cancer maintains without these accessory food factors.

12 The functioning organism is evidently the result of an environment rich in growth stimulating substances supplied by preexisting lower growing and nonfunctioning forms.

13 In cancer the cell becomes independent. The cells in the aging organism must tend to revert to this state. Any tissue may be made cancerous by anything that frees the cells of the effect of their intercellular substances and an active circulation of blood.

THE VALUE OF IRON IN ANEMIA

AN EXPERIMENTAL STUDY *

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AND

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The question of the absorption and utilization of iron in the formation of hemoglobin has long engrossed the attention of clinicians and physiologists. Unfortunately the results of these researches are far from being in accord in spite of the great amount of labor expended.

If we leave out the earlier work as for the most part too inaccurate to be of service, the modern study begins with Bunge,¹ under whose direction Haeusermann studied the question exhaustively. His plan was to feed growing animals food low in iron, generally rice and milk, half of the animals receiving in addition medicinal iron (ferric chloride). After several months' treatment, the animals were killed and the total hemoglobin determined, and in some of the animals the total iron also. He found that the animals became highly anemic on rice and milk, and that those receiving iron in addition contained no more hemoglobin than the controls. Furthermore, the animals receiving iron had no more total iron in their bodies, except in one experiment. His results on rabbits and dogs were discordant, for after one or two months' feeding he could not determine whether iron was not stored up through lack of absorption or absorbed and then eliminated.

Abderhalden,² in an extensive series of researches, came to the conclusion that animals on an iron-poor diet plus medicinal iron failed to produce as much hemoglobin as animals on a diet rich in food iron. On the other hand his observations led him to the view that medicinal iron in some way stimulated, for short periods of time at least, the formation of hemoglobin from the food iron. Tartakowsky,³ however, came to the conclusion that medicinal inorganic iron is both absorbed and utilized in hemoglobin formation. It is worthy of note that his young animals fed on rice and milk became anemic and finally ceased to grow but that medicinal iron added to their diet caused the blood to gain in hemoglobin and the animals to grow again. He considers that Abderhalden's results should likewise be interpreted in this sense. Schmidt⁴ came to substantially the same conclusions as Tartakowsky.

* From the Department of Medicine of the University of Illinois College of Medicine

1 Bunge. *Physiological Chemistry*, Chapter 25

2 Abderhalden. *Physiological Chemistry*, Ed 3, Chapter 35

3 Tartakowsky. *Arch f d ges Physiol* **100** 586, 1903, *ibid* **101** 423, 1904

4 Schmidt. *Verhandl d Deutsch path Gesellsch* **15** 91, 1912

In criticism of this work, it seems quite clear that many of the animals were on an inadequate diet, not only in respect to vitamins but also in some cases in respect to proteins and fat as well. Many of their results were certainly due to deficiency diseases rather than to the lack of iron only.

Recently there has been a revival of interest in the subject. Whipple⁵ and his associates have studied the question anew and along somewhat different lines. They have shown that the diet of the animals was of the very greatest importance, and their experiments have given no support to the value of inorganic iron in simple anemias. Musser⁶ also has reported some experiments which failed to show any favorable influence of inorganic iron in experimental hemorrhagic anemias. On the other hand, Scott and Barcroft⁷ state that iron seems to "anticipate the cure, which occurs normally." Gibson and Howard,⁸ while admitting the superiority of a diet rich in food iron, say, "We still feel justified in continuing the administration of iron in the form of Bland's pills." From this it may be inferred that these authors consider inorganic iron as of value, or at any rate that the contrary has not been satisfactorily demonstrated.

It seemed to us that, in view of the discordant results obtained by different authors, the subject should be reinvestigated, with the emphasis laid on the following points: (1) to select an animal that can be obtained in large numbers in large litters, the individuals of which are practically identical, (2) to use these animals in sufficient numbers to eliminate the varying results, which must necessarily obtain when only a dozen or two are used, (3) to use a standard basal diet adequate in all respects, (4) to continue the experiments over a time sufficiently long to ensure decisive results, and (5) in determining the hemoglobin, to use a method of the highest possible degree of accuracy and free from the defects of the usual colorimetric methods.

To comply with the first point, we selected the rat, because its life history is thoroughly known, and we secured all our animals, with the mothers, when only a few days old. All were of the same breeding. To cover the second point, we used several hundred animals, 386 rats and thirty-two dogs. The diet used was the standard casein diet of Osborne and Mendel, yeast being added to carry vitamin B, and on this our rats

5 Whipple, G. H., Robscheit, F. S., and Hooper, C. W. *Blood Regeneration Following Simple Anemia*, *Am J Physiol* **53** 151-263 (Sept.) 1920.

6 Musser, J. H., Jr. *Influence of Inorganic Iron on Regeneration of Blood After Hemorrhagic Anemia*, *Arch Int Med* **28** 638 (Nov.) 1921.

7 Scott and Barcroft. *Biochem J* **18** 1, 1924.

8 Gibson, R. B., and Howard, C. P. *Metabolic Studies in Pernicious Anemia*, *Arch Int Med* **32** 1 (July) 1923.

grew and reproduced normally. The iron-free diet was obtained by simply leaving out the ferrous lactate from the salt mixture used in making up the foregoing diet. As thus constituted, analysis showed the iron content to be 0.072 mg per hundred grams of food. While for convenience we speak of this as the iron-free diet to distinguish it from the iron-containing diet, to which ferrous lactate has been added, it is in reality only an iron-poor diet. To satisfy the fourth point, most of our experiments were continued over several months and always until satisfactorily uniform results showed that a longer continuance would be productive of no further changes. Some of the experiments were continued as long as eight months. To comply with the fifth point, we chose the spectrophotometric method of determining the hemoglobin values, since according to Buerker's investigations, it is the most accurate for this purpose. A further reason for choosing this method was that one of us (C. S. W.) had previously conducted an extensive research based on this method, and was therefore thoroughly familiar with the technic and various phases of spectrophotometric investigations.

PRINCIPLES INVOLVED IN QUANTITATIVE SPECTROPHOTOMETRIC DETERMINATION OF HEMOGLOBIN

Without discussing this method in detail, we shall give briefly the salient points, assuming that the basic principles are already known. The method is based on the fundamental law that for each colored substance the ratio, concentration to extinction coefficient, is a constant. This law is generally expressed $C/E = A$. In our case the concentration C is, of course, the number of grams of oxyhemoglobin in 1 c.c. of the solution examined. The extinction coefficient is the reciprocal of the thickness of solution required to reduce light of the intensity one to the intensity one-tenth. In the spectrophotometer in question (we used the improved Huefner apparatus), the thickness of the layer of solution remains constant, and E is determined by measuring the intensity of the remaining light. This is effected by rotation of the analyzing with respect to the polarizing Nicol. Now it can be mathematically shown that the extinction coefficient, E , is equal to $-2 \log \cos O$, where O is the angle of rotation just referred to. In order to obtain E , we measure the angle of rotation, O , necessary to produce equality in the upper and lower fields. This angle is read off on a vernier and from this the extinction coefficient, E , is computed, either from a table of logarithms or more conveniently from a logarithmic curve that Buerker has constructed for this purpose.

Since we are always dealing with a solution of one and the same substance, oxyhemoglobin, the concentrations are directly proportional to

their respective extinction coefficients, so that the latter are used as a convenient method of expressing relative concentration. The figures given in our tables are these extinction coefficients obtained in the manner described. If it is desired to convert these into absolute values of hemoglobin, all that is necessary is to multiply these extinction coefficients by a factor known as the absorption ratio, of course making due allowance for the dilution of the solution.

The construction of the apparatus is such that readings of the vernier are taken in two different sectors of the circle, obtained by rotating the prism first to the right and then to the left. Since any lack of adjustment of the apparatus would immediately be made manifest by the failure of these opposed readings to agree, it is obvious that we have here a guarantee of the proper functioning of the apparatus. A minimum of four readings was taken for each determination, and if an extra degree of accuracy was desired ten readings were taken. Much experience showed that if a number of determinations were made on the same day, no perceptible increase of accuracy was obtained with a larger number of readings because of the increased eye fatigue. We elsewhere have discussed the average and probable error of reading and have shown that it was negligibly small. We had a special dark room at our disposal and the entire apparatus was enclosed in a large, hinged, light proof case, so constructed that no extraneous light could reach the eye.

When we consider the high degree of accuracy in the actual reading of the vernier of the apparatus, it is obvious that the error involved in measuring and diluting the blood is likely to be several times as great as the error in reading. Our pipet was an especially constructed and calibrated piece of heavy thermometer tubing with thick walls, delivering 0.03865 gm., or 0.03646 c.c., of blood. This amount may be readily obtained either by a prick of the vein in a dog's leg or by snipping off a bit of a rat's tail. In actual practice we proceeded as follows. Into long necked, glass stoppered flasks of 50 c.c. capacity were measured 4 c.c. of a 0.1 per cent sodium carbonate solution, using a Mohr 5 c.c. normal pipet. A pipetful of blood was then blown into this and the solution thoroughly agitated, which gave a perfectly clear, transparent fluid. Our pipet was graduated as a delivery pipet, and by blowing with force each time for ten seconds and withdrawing the tip of the pipet from the fluid while still blowing, a high degree of uniformity was obtained. This is shown from the accuracy determinations below. The blood solution was then transferred to the Troegnen and the readings taken as described. A special feature of the spectrophotometer is that by taking a second reading between the bands any deterioration or alteration of the blood can be instantly determined and the specimen discarded.

ACCURACY DETERMINATION

To test directly the precision obtained in practice, a number of accuracy determinations were made from time to time of which two typical ones are given here. A pipetful of blood was taken from a rat's tail, diluted in the manner described with 4 c c of sodium carbonate solution, and the pipet cleaned and dried. A second pipetful of the same blood was then taken and diluted in the same way. These were then examined spectrophotometrically with the results given in Tables 1 and 2.

Since the concentrations are proportional to the extinction coefficients, it will be seen that with only two readings on each side of the vernier a high degree of accuracy is obtained.

TABLE 1—*Accuracy Determination of Solutions of Same Concentration*

Solution A		Solution B	
Left	Right	Left	Right
70.2	70.3	70.4	70.2
70.4	70.1	70.3	70.9
140.6	140.4	140.7	141.1
Average of readings	70.25	Average of readings	70.45
Extinction coefficient	0.943	Extinction coefficient	0.951

TABLE 2—*Accuracy Determination of Solutions of Different Concentrations*

Solution A		Solution B	
Left	Right	Left	Right
73.4	73.0	75.6	75.3
73.0	73.3	75.8	75.8
73.3	73.3	75.7	75.6
73.1	73.2	75.4	75.7
73.1	73.3	75.6	75.4
365.9	366.1	378.1	377.8
Average of readings	73.2	Average of readings	75.59
Extinction coefficient	1.078	Extinction coefficient	1.208

In another test we proceeded as follows. One pipetful of blood (A) was blown into 4 c c of sodium carbonate, a second (B) into 4.5 c c of the same fluid. A simple calculation shows that the concentrations in these two cases are to each other 124.42/110.71. The two solutions were then examined, the results are given in Table 2.

Comparing these ratios, we find that $124.42/110.71 = 1.124$ and $1.208/1.078 = 1.120$.

For ease in the interpretation of these and subsequent figures, it is convenient to bear in mind that a difference of 0.01 in the extinction coefficient represents a difference of less than 1 per cent in the amount of hemoglobin. In other words, the two solutions were so prepared that their concentrations were 1.124, while the concentrations as actually determined were 1.120, a difference of only a small fraction of 1 per cent.

It must be remembered that while the vernier readings in degrees of an arc are given to show what is actually read, the concentrations of the solutions are not proportional to these but to the extinction coefficients, i e, to $-2 \log \cos$ of these arcs. Since these accuracy determinations were made in the ordinary course of the work, they represent the degree of accuracy actually attained and not that which is merely theoretically possible. These demonstrate very clearly the correctness of Buerker's statement as to the exceedingly great accuracy of the method, especially for the comparative determination of hemoglobin, and, of course, since no color comparisons are made there is no standard to change or deteriorate, which is regularly the case with most colorimeters. In addition it has the very great advantage of recognizing at once any deterioration of the solution, such as may result from a slight admixture of alcohol or ether in the cleaning of a pipet, or from long standing. For a fuller discussion of the method as applied to hemoglobin determination, reference should be made to our previous work.⁹

The method used in the determination of iron in the tissues was that of Ripper-Schwarzer.

To summarize, our method of attacking the problem, keeping in mind the foregoing five points, was

- 1 To administer medicinal iron orally to rats, with suitable controls, in other groups to administer iron subcutaneously, and to compare the amount of hemoglobin in the blood and tissues of these with a group of noninjected controls

- 2 To repeat these experiments in rats rendered anemic by single or multiple bleedings

- 3 To determine the effects on the blood of young rats of medicinal iron administered to the mothers during the periods of gestation and lactation

- 4 To control the results of the foregoing by similar experiments on a different type of animal (dog), as well as to study the effects of intravenous injections of iron, for which purpose the rat is ill adapted

Our division of work was such that one of us (C S W) carried out all the spectrophotometric work, the other (H N E) carried out all the iron determinations and assumed the entire charge of the animals.

EXPERIMENTS ON RAT GROUP 1 (FORTY FIVE RATS)

A group of forty-five rats from five litters was divided into two groups half of each litter placed on our standard iron-free diet, and half on the iron-containing diet already described. They were kept on these

⁹ Williamson, C S. Influence of Age and Sex on Hemoglobin, Arch Int Med **18** 505-528 (Oct) 1916

two diets from March 5 to September 24, about six and one-half months. Several hemoglobin determinations were made on dates between these extremes, but to simplify the tables and to save space these are not given. They showed, however, no significant change. It may be stated here to apply to this group as well as to the other subsequent rat groups, that the weights pursued a perfectly normal course, that the animals remained lively and healthy, and that pairs selected at random bred in a normal manner with an average number of healthy young. The results are given in Table 3.

TABLE 3—Hemoglobin Concentration in Rat Group 1

On Iron Free Diet			On Iron Containing Diet		
Rat	March 5	September 21	Rat	March 5	September 21
2	1.031	1.100	1	1.081	1.207
4	1.000	1.095	3	1.141	1.078
6	1.082	1.068	5	1.073	1.162
8	1.101	0.974	7	1.021	1.146
10	1.167	1.153	11	1.148	1.187
12	1.018	1.098	13	1.127	1.215
14	1.023	1.213	15	1.032	1.199
16	1.086	1.066	17	1.050	1.213
18	1.044	1.078	19	1.101	1.127
20	0.950	1.062	21	1.090	1.078
22	1.024	1.041	23	1.062	1.092
24	1.161	1.157	25	1.122	1.135
26	1.031	1.093	27	1.048	1.015
28	1.124	0.834	29	1.103	1.071
30	1.182	1.090	31	1.103	1.214
38	1.109	1.142	33	1.063	1.092
40	1.134	1.063	35	1.077	1.017
40B	0.923	1.125	37	1.062	1.076
42	1.160	1.166	40A	1.156	1.037
44	1.093	1.088	41	1.103	1.059
46	1.148	1.244	43	1.097	1.093
48	1.075	1.129	45	1.045	0.996
			49	1.019	1.261
Average	1.073	1.098	Average	1.083	1.123

TABLE 4—Averages in Group 1

Iron free diet	75.5 mg per kilogram of body weight
Iron diet	87.1 mg per kilogram of body weight

It will thus be seen that while each group gained one or two points in hemoglobin concentration, the differences are well within the limits of natural variation and experimental error. After the last determination, the rats were killed, the entire digestive tract was removed, and the iron content of the entire rat determined by the method described. The average results of the iron analyses are given in Table 4.

We see, therefore, that while the bodies of the rats that received iron contained more iron than the controls on an iron-free diet, this in no wise affected the hemoglobin concentration. The iron is stored in the body, but seems not to be capable of conversion into hemoglobin nor does it in any way stimulate the production or mobilization of iron from any possible reserve supply that may exist.

RAT GROUP 2 (THIRTY SIX RATS)

In this group the experimental conditions that we had in Group 1 were repeated. The animals were kept on their respective diets four and one-half months.

A study of Table 5 shows that the group on an iron-free diet had a hemoglobin concentration of 1.061 at the beginning and 1.05 at the end of the experiment, while the group on an iron-containing diet had concentrations of 1.105 and 1.106 at these times. In other words, the changes in both groups were negligible in amount.

In this group, as in Group 1, we have the same increase in total body iron. It would seem evident that the iron must remain in the body for

TABLE 5—*Hemoglobin Concentration in Rat Group 2*

On Iron Free Diet			On Iron Containing Diet		
Rat	May 3	September 21	Rat	May 3	September 21
51	1.055	1.184	50	1.123	1.170
55	1.111	1.025	52	1.152	1.093
57	1.152	0.978	54	1.110	1.138
59	1.131	1.144	56	1.228	1.187
61	1.215	1.230	58	1.113	1.114
65	1.110	1.006	60	0.970	1.049
69	1.087	1.038	64	1.183	1.032
71	0.985	1.046	66	1.242	1.111
73	1.087	1.118	68	1.167	1.220
75	1.110	1.005	70	1.100	1.177
77	0.764	0.872	74	1.063	1.117
83	0.982	0.968	76	1.111	1.018
91	1.071	0.533	82	1.078	1.073
93	1.090	1.046	86	1.041	1.117
95	0.995	0.944	90	1.052	1.043
97	1.008	1.003	92	1.101	1.159
99	1.083	1.296	94	1.051	1.124
			96	1.085	1.130
			98	0.971	1.132
Average	1.061	1.050	Average	1.105	1.106

TABLE 6—*Averages in Iron Analyses in Group 2*

Iron diet	73.6 mg per kilogram of body weight
Iron-free diet	39.5 mg per kilogram of body weight

some time, since the increase is much too great to be accounted for except on that assumption.

RAT GROUP 3 (EIGHTY FIVE RATS)

As in the two previous groups, the rats were kept on the two diets for a little over four months. These rats were chosen as young as they could be handled successfully, i. e., about three or four weeks after weaning. At this time, as in the human infant after the second year, the normal hemoglobin curve is rising.

It will be seen that both groups gained in hemoglobin concentration in the normal manner and that the averages are almost identical.

The rats receiving iron gained 0.9, and the controls on an iron-free diet gained 0.1. Here, again, in young animals the oral administration of

iron fails to increase the rate of gain in the slightest degree. The large number of healthy young rats used makes an average of considerable accuracy.

After the last analysis, the rats were killed and the livers and spleens analyzed with the results given in Table 8.

TABLE 7—Hemoglobin Concentration in Rat Group 3

On Iron Free Diet			On Iron Containing Diet		
Rat	June 5	October 10	Rat	June 5	October 10
101	0.999	1.159	100	1.100	1.046
103	1.001	1.118	102	1.086	1.068
105	1.138	1.180	104	1.081	1.065
107	1.036	1.081	106	1.010	1.041
110B	0.957	1.098	108	1.034	1.058
111	1.019	1.018	110	0.987	1.026
113	0.979	1.076	110A	0.983	0.948
115	1.008	1.044	112	0.964	1.021
117	0.958	1.075	114	1.067	1.068
119	0.986	1.068	116	1.056	1.053
121	0.961	1.103	118	1.027	1.041
123	1.063	1.045	124	0.968	1.190
127	0.985	1.272	132	0.975	1.116
131	1.035	1.133	136	1.021	1.146
135	0.933	1.206	140	0.986	1.066
141	1.001	1.001	140A	1.130	1.127
143	0.949	1.061	142	1.031	1.114
145	1.052	1.200	144	0.983	1.263
147	1.001	1.177	146	1.000	1.125
149	1.044	1.419	148	1.111	1.100
150B	0.977	1.073	150	0.912	1.083
151	0.959	1.098	150A	0.934	1.088
153	0.924	1.138	150C	0.983	1.194
155	0.992	1.044	152	1.071	1.146
157	0.970	1.165	154	0.895	0.820
160B	1.071	1.098	156	1.020	1.170
160C	0.999	1.144	158	1.013	1.133
161	1.068	0.912	160	1.049	1.248
165	1.029	1.168	160A	0.866	1.036
167	1.046	1.092	162	1.028	1.124
169	1.046	0.739	164	1.026	1.245
173	0.964	1.050	166	0.995	1.347
175	0.927	1.022	168	1.040	1.164
181	0.897	1.280	170	1.008	1.093
183	1.006	0.866	172	0.935	1.187
185	1.068	1.029	174	0.968	1.053
187	1.061	1.167	176	0.848	1.149
190B	1.143	1.155	182	1.041	1.008
193	0.811	1.151	184	1.035	1.103
197	1.022	1.138	186	1.100	1.339
199	0.987	1.164	188	0.948	1.103
			190A	1.148	1.177
Average	1.002	1.106	194	1.004	0.681
			196	1.067	1.048
			Average	1.012	1.100

TABLE 8—Averages in Iron Analyses in Group 3

Iron-Free Diet		Iron Diet	
	Iron per 100 Gm of Tissue		Iron per 100 Gm of Tissue
Livers	28.0 mg	Livers	29.6 mg
Spleens	155.0 mg	Spleens	169.0 mg

These differences are relatively slight, and suggest that iron, in comparatively small quantities given in this way by mouth, while it remains in the body a considerable time, is not stored up in the liver and spleen in any great measure, probably owing to the fact that it is again excreted during the months the feeding is being carried on.

It is evident by comparison of these figures with those obtained for the iron content of the whole rat (in Groups 1 and 2) that some but not all of the iron is contained in the spleen and liver. A rat spleen averages 0.8, a liver 2.9, and the total blood about 6.2 mg of iron.

RAT GROUP 4 (TWENTY EIGHT RATS)

Young rats were bled (28 per cent of their blood volume), then divided into two groups, one being placed on an iron-free and the other on an iron-containing diet. The hemoglobin concentration was estimated at short intervals. In less than eight weeks, the hemoglobin concentration of the blood had become stationary and was at the same level as previous to the bleeding. (To save space the original and intermediate

TABLE 9—Hemoglobin Concentration in Rat Group 4*

Rats on Iron-Free Diet†				Rats on Iron Containing Diet‡			
Rat	Feb 25, 1924	Feb 27, 1924	April 4, 1924	Rat	Feb 25, 1924	Feb 27, 1924	April 4, 1924
1	1.124	Bled	1.230	0	1.093	Bled	1.015
3	1.199	(12 per cent)	1.083	2	0.977	(12 per cent)	1.166
5	1.046		1.168	6	1.068		1.199
9	1.117		1.133	14	1.135		1.193
11	1.111		1.174	18	0.970		1.141
15	1.043		1.177	20	1.081		1.188
19	1.135		1.119	24	1.088		1.073
21	1.133		1.083	26	0.984		1.038
23	0.989		1.151	30	1.051		1.072
25	1.117		1.132	32	1.144		1.149
31	1.126		1.166	36	1.125		1.080
33	1.076		1.083	44	1.091		1.129
35	1.108		1.152				
37	1.114		1.174				
43	1.076		1.152				
45	1.134		1.156				
Average	1.103		1.145	Average	1.151		1.114

* When the rats were bled, they were first etherized and the blood was then drawn directly from the heart. The blood volumes were estimated from the figures of Scott and Barcroft, i. e., 6.28 c.c. of blood per hundred grams of rat.

† Average hemoglobin concentration before bleeding, 1.117. Rats first bled (28 per cent) Nov. 27, 1923.

‡ Average hemoglobin concentration before bleeding, 1.113. Rats first bled (28 per cent) Nov. 27, 1923.

readings are not given in Table 9). The original average in the iron-free group was 1.117, and in the iron group, 1.113. The rats were then bled (12 per cent of the blood volume) and a hemoglobin determination was made. Five weeks later the hemoglobin had again attained its original value. A glance at Table 6 shows that after the first bleeding the hemoglobin concentrations were identical in the two groups, the figures being 1.103 and 1.151. Five weeks after the second bleeding the same statement held true, the concentrations were 1.145 and 1.114. The group receiving iron in the diet was, if anything, a trifle lower, the rats on an iron-free diet gaining 0.04, those on an iron-containing diet losing 0.04.

In this group the hemoglobin values were not in the least influenced by the administration of medicinal iron. It may be noted that under our

dietary conditions the rapidity with which the hemoglobin values of the blood regained their original values after bleeding was very much greater in the rat than in the dog. After the last determination the animals were killed, and the iron contents of the livers and spleens was determined.

The increase, even though small, in the amount of iron in the livers and spleens of the group receiving iron in the diet makes it clear that the fault lies not with the absorption of the iron but with its failure to be converted into hemoglobin. That the increase is relatively small in animals receiving iron in small quantities and by mouth is not strange,

TABLE 10—*Averages in Iron Analyses in Group 4*

Iron Free Diet		Iron Diet	
	Iron per 100 Gm of Tissue		Iron per 100 Gm of Tissue
Livers	48.4 mg	Livers	52.6 mg
Spleens	138.0 mg	Spleens	147.0 mg

TABLE 11—*Hemoglobin Concentration in Rat Group 5 A (Forty-One Rats)*

On Iron-Free Diet				On Iron Containing Diet			
Rat	Before Bleeding, March 11		June 5	Rat	Before Bleeding, March 11		June 5
50	1.105	Bled	1.072	50A	1.020	Bled	1.129
55	1.083	twice	1.085	50B	1.039	twice	1.148
59	1.141	30 per	1.013	52	1.236	30 per	1.202
60A	1.191	cent	1.116	56	1.098	cent	1.105
61	1.001	March 17,	1.110	58	1.203	March 17,	1.068
63	1.003	and	1.113	60	1.101	and	1.215
69	1.040	25 per	0.929	60B	1.090	25 per	1.078
71	1.171	cent	1.199	62	1.146	cent	1.148
73	1.090	May 5	1.117	64	1.135	May 5	1.174
75	1.106		1.076	66	1.088		1.263
77	1.153		0.829	68	1.083		1.171
80A	1.112		1.113	71	1.185		1.203
80D	0.988		0.936	76	1.296		1.130
83	1.125		1.099	78	1.223		1.228
85	1.086		0.942	80	1.167		1.146
89	0.916		1.117	88	1.072		1.206
90A	1.108		1.129	90	1.174		1.180
90E	1.191		1.083	90C	1.137		1.173
93	1.092		1.050	90F	1.063		1.081
95	1.203		1.080	91	1.106		1.194
				98	1.109		0.972
Average	1.100		1.060	Average	1.132		1.153

since the amounts are small and the time during which it may be exerted relatively long, as compared with the large doses and shorter times of injection in the groups receiving iron subcutaneously.

RAT GROUP 5 (FORTY ONE RATS)

In a group of forty-one rats, the hemoglobin concentration was first taken, following which 2 c.c. of blood was removed from the heart. As the average weight was 85 gm., this represented 30 per cent. of the total blood volume. Half the animals were placed on iron-free and the other half on an iron-containing diet, and approximately five weeks later the

hemoglobin concentration was again determined. The two groups were found to be substantially identical. To save space, these intermediate figures are omitted. The rats of the foregoing groups were then bled again (25 per cent of the blood volume), since on reflection it seemed highly probable that the reserve stores of iron in the liver, spleen, etc., would hardly be exhausted by a single bleeding. Thus it might be thought that medicinal iron may not be absorbed and utilized when only a moderate degree of anemia exists, and the liver and spleen contain a more available form of iron, yet medicinal iron may be absorbed and utilized when a severe anemia exists and iron reserves of the liver and spleen are greatly depleted.

One month after the second bleeding when the hemoglobin was again determined, the group on an iron-free diet was found to be 0.04 lower than at the beginning (1.1 and 1.06), while in the group on an iron-containing diet, the figures were 1.132 and 1.53, respectively. In

TABLE 12—Hemoglobin Concentration in Rat Group 5 B (Twenty-Eight Rats)

On Iron Free Diet				On Iron Containing Diet			
Rat	Before Bleeding,		July 23	Rat	Before Bleeding,		July 23
	March 11				March 11		
55	1 083	Bled	1 146	50B	1 039	Bled	1 127
59	1 141	28 per	1 119	52	1 236	28 per	1 135
67	1 122	cent	1 144	56	1 098	cent	1 119
69	1 070	July 10,	1 086	60	1 101	July 10,	1 062
71	1 171	had	1 093	60B	1 090	had	1 046
73	1 090	been	1 111	62	1 146	been	1 174
75	1 106	previ	1 109	64	1 135	previ	1 096
77	1 153	ously	1 101	66	1 088	ously	1 106
80D	0 988	bled	0 990	74	1 185	bled	1 101
83	1 125	twice	1 093	76	1 296	twice	1 166
85	1 087		1 068	78	1 223		1 073
90E	1 191		1 053	80	1 167		1 051
95	1 203		1 049	90	1 174		1 101
				90E	1 137		1 066
				94	1 106		1 063
Average	1 118		1 089	Average	1 145		1 095

other words, we may conclude that after two bleedings of considerable size the animals on a diet containing iron showed essentially the same hemoglobin values as the control group with no iron.

To check further this result, a third bleeding was performed (28 per cent of the blood volume) on most of the rats, and the hemoglobin readings were taken two weeks later. As will be evident from Table 12, the last average readings are substantially identical in the two groups. In the group on an iron-free diet, the hemoglobin concentration was 1.118 before the first bleeding, and 1.089 after the last, while in the group receiving iron the corresponding figures are 1.148 and 1.095, respectively. From these figures it is evident that even after three bleedings, aggregating 62.2 per cent of the blood volume, the hemoglobin in the blood regained its normal value within a few months, and that the administration of medicinal iron in no wise facilitated this recovery.

The rats were then killed and the iron content of the livers and spleens determined

It will be seen that while the hemoglobin concentration remained practically unchanged the iron content of the livers and spleens, particularly the latter, showed a considerable increase. The iron was absorbed to a greater or less degree but not converted into hemoglobin

TABLE 13—*Averages in Iron Analyses in Group 5 B*

Iron Diet		Iron-Free Diet	
	Iron per 100 Gm of Tissue		Iron per 100 Gm of Tissue
Livers	41.0 mg	Livers	37.2 mg
Spleens	112.0 mg	Spleens	71.0 mg

TABLE 14—*Hemoglobin Concentration in Rat Group 6*

On Iron Free Diet			On Iron Free Diet Plus Iron Given Hypodermically		
Rat	February 9	March 15	Rat	February 9	March 15
OA	1.044	1.056	D	0.964	1.068
OB	1.046	0.994	1	1.171	1.080
OC	1.223	1.143	3	1.004	1.101
4	1.155	1.010	5	0.963	1.138
6	1.025	0.938	7	1.032	0.951
8	0.992	1.049	9	0.989	0.995
9	0.989	0.995	10A	0.938	1.015
20	1.090	1.060	10B	1.050	1.049
10A	1.096	1.149	10D	0.969	1.031
20B	1.028	1.098	10F	1.096	1.028
20C	1.077	1.199	11	1.004	1.010
20D	1.046	1.152	14	1.075	0.992
101	1.071	1.092	15	1.024	1.018
21	1.031	1.125	16	1.051	1.051
22	1.010	1.056	18	1.001	1.055
23	1.073	1.138	19	1.013	1.108
24	1.010	1.092	30	0.970	1.020
25	1.021	1.111	30A	1.073	1.020
26	1.049	1.185	30B	1.000	0.940
27	1.049	1.145	30I	1.033	0.940
28	1.034	1.051	31	1.078	1.044
29	1.096	1.097	33	1.093	1.028
40	1.027	0.947	35	0.981	1.033
40A	1.083	1.174	36	1.102	1.091
40C	0.961	1.063	37	1.156	0.947
40D	1.039	1.043	38	0.956	1.006
40E	1.043	1.028	39	1.041	1.066
41	1.018	1.025			
42	0.961	0.949	Average	1.031	1.030
43	0.965	1.077			
44	1.050	1.017			
45	1.045	1.086			
46	1.051	0.949			
47	1.038	1.247			
48	1.038	1.070			
Average	1.045	1.075			

RAT GROUP 6 (SIXTY TWO RATS)

In Group 6 the intention was to test the value of medicinal iron used hypodermically. Young rats were chosen, their concentration was determined, and they were then divided into two groups, both on an iron-free diet, but one group receiving 0.5 c.c. of a 1 per cent solution of iron and ammonium citrate subcutaneously three times a week. These

diets and injections were continued for five weeks during which hemoglobin determinations were made several times. As the intervening determinations showed nothing of significance, only the first and last determinations are given in Table 14.

It is evident from an inspection of the figures in Table 14 that the group receiving injections remained exactly stationary at 1 031 and 1 03, while the control (noninjected) group gained a mere trifle, reaching 1 045 and 1 075. These results demonstrate that with all rats on an iron-free diet medicinal iron, hypodermically given, in no wise increases the hemoglobin concentration. As a matter of fact, we have shown in other rats that the long continued use of iron in this way and in this dose produces a distinct deleterious effect, the rats become sick, do not increase normally in weight, and many die.

TABLE 15—*Averages in Iron Analyses in Rat Group 6*

Injected Rats		Noninjected Rats	
Livers	Iron per 100 Gm of Tissue	Livers	Iron per 100 Gm of Tissue
Spleens	71.6 mg	Spleens	22.3 mg
	105.0 mg		85.2 mg

TABLE 16—*Comparative Hemoglobin Concentrations in Young of Rats on Iron-Free Diet and on Iron-Containing Diet*

Dams on Iron-Free Diet		Dams on Iron-Containing Diet
Extinction Coefficient	Extinction Coefficient	Extinction Coefficient
1 058	0 999	1 093
1 084	1 024	1 041
1 081	0 975	1 068
0 997	1 017	1 073
1 106	0 970	1 086
1 081	1 004	1 188
1 027	0 934	0 887
0 994	0 999	1 109
		1 061
Average	1 022	1 059

After the last determination of hemoglobin, the animals were killed and the iron content of the livers and spleens was determined. The results are given in Table 15.

As might have been expected with relatively large doses given subcutaneously, the storage in the liver and spleen was very great, yet there was not the slightest evidence of its utilization as hemoglobin.

RAT GROUP 7 (SIXTY ONE RATS)

In this group an attempt was made to determine whether the young of rats that had been kept on an iron-free diet during the entire period of gestation and lactation would have a lower hemoglobin concentration than those born of dams that had been kept on such a diet plus medicinal iron during the same period.

To eliminate any possible effect of age, the blood was taken in all cases at about the twelfth day of life. In all, sixteen young from three dams on an iron-free diet and nine young from two dams on an iron-containing diet were studied. The results are shown in Table 16.

In view of the individual concentrations of hemoglobin, these results are essentially identical, or at least within the margin of variation found in different groups of animals. To make this point clear, we append a table of two groups of animals of exactly the same age whose dams were on a general mixed diet. As will be seen, the average of the first group is 1.061, that of the second 1.105, a difference of a little less than 4 per cent, which is approximately the difference between the two groups under discussion.

TABLE 17—*Hemoglobin Concentrations in Animals on General Mixed Diet*

Rat	Extinction Coefficient	Rat	Extinction Coefficient
1	1.055	1	1.123
2	1.111	2	1.152
3	1.152	3	1.110
4	1.131	4	1.228
5	1.215	5	1.113
6	1.110	6	0.970
7	1.087	7	1.183
8	0.985	8	1.242
9	1.087	9	1.167
10	1.110	10	1.160
11	0.764	11	1.063
12	0.982	12	1.111
13	1.071	13	1.078
14	1.090	14	1.041
15	0.995	15	1.052
16	1.008	16	1.101
17	1.083	17	1.051
		18	1.085
Average	1.061	19	0.971
		Average	1.105

The conclusion would seem justified that the administration of medicinal iron to pregnant rats does not increase the hemoglobin concentration in the blood of the offspring.

EXPERIMENTS ON DOGS

Healthy adult dogs were selected and placed on a maintenance diet of white bread and whole milk. This is a rather iron-poor, but by no means an iron-free diet, since milk contains, according to Sherman, 0.24 mg. of iron per hundred grams and white bread 0.90 mg. per hundred grams. In one group meat was added to test the difference of the behavior of the hemoglobin on the two diets. In this case half the bread was withdrawn, and an equal number of calories of meat were added. In other respects, the methods were essentially the same. Blood was obtained by a prick with a sharp cataract knife from the vein of the hind leg, and intravenous injections, when used, were given in the corresponding vein of the opposite leg. In all cases more than sufficient

time was allowed to elapse between successive hemoglobin determinations to permit the blood volume to return to the normal. Water was given freely and at all times.

Various authors have called attention to the fact that the dog is not a suitable animal for this type of work, a statement with which we are in entire accord. Our work is essentially based on the results of our rat experimentation, the dogs being used for intravenous injections, for which the rat is not suitable, and with the idea of forming a control in a different type of animal.

The dogs were made anemic by bleeding them from the heart, after etherization. The volume removed was calculated from the figures of Whipple, Robscheit and Hooper.

DOG GROUP 1 (FIVE DOGS)

Large dogs were bled directly from the heart, the amount taken being 25 per cent of the blood volume. In order to determine whether the anemia produced was actually as great as was postulated from the

TABLE 18—*Hemoglobin Concentration in Dog Group 1*

	Dog	Before Bleeding	Octo- ber 24	Novem- ber 2	Novem- ber 15	Novem- ber 30	Decem- ber 12
Noninjected controls	4 8	1 046 0 931	Bled 25 per cent of blood volume	0 783 0 654	1 067 1 098	1 015 0 970	0 949 0 970
Average		0 989		0 719	1 083	0 993	0 960
Injected dogs	3 5 7	1 119 1 236 1 251			1 277 0 927 0 897	1 357 1 233 1 011	1 330 1 096 0 976
Average		1 202			1 034	1 204	1 201

amount of blood withdrawn, additional determinations of hemoglobin were made on two of the dogs, Nov. 2, 1923. The bleeding had been done Oct. 24, 1923. The results were as follows. Before bleeding, the concentrations in the blood were 0.997 and 1.087, respectively, and on the second date 0.654 and 0.783, respectively. This is to be interpreted as showing that the blood volume had come back to approximately the normal and also shows that the anemia, as measured by the concentration of hemoglobin, was approximately what it had been postulated to be. All the animals were on a bread and milk diet.

The figures given for Dogs 4 and 8 show that from October 24 to November 2 (nine days) the hemoglobin concentration was reduced from 0.989 to 0.719 (about 27 per cent), showing that the blood volume was fully restored to normal in that time. We have made numerous similar observations. After a week or ten days, the regenerative effects are rapidly manifested. The figures for November 15 should be noted. The injected group received 1.5 cc of the iron and ammonium citrate 1 per cent solution subcutaneously three times a week for five weeks.

It will be seen from an inspection of Table 18 that in the six weeks that elapsed from the date of bleeding the control dogs had regained their original hemoglobin concentrations. The injected animals showed no difference in the behavior of their blood from that shown by the noninjected controls, their concentration before bleeding being 1 202, and six weeks later 1 201.

We may conclude, therefore, that after a single large hemorrhage medicinal iron given subcutaneously does not enable the animal to regain a previous concentration of hemoglobin either more quickly or more efficiently.

At the end of the experiment the dogs were killed, and the amount of iron in the livers, spleens and bone marrows was determined. Table 18 shows the average figures obtained.

The great increase in the amount of iron in the spleens of the injected group is worthy of note. It is again evident that the failure to increase

TABLE 19—*Averages in Iron Analyses in Dog Group 1*

	Noninjected Controls	Injected Dogs
Livers	25.5 mg per 100 gm of tissue	30.0 mg per 100 gm of tissue
Spleens	162.2 mg per 100 gm of tissue	297.3 mg per 100 gm of tissue

TABLE 20—*Averages in Iron Analyses in Bone Marrow of Dogs*

Noninjected Controls		Injected Dogs	
Dog 4	514	Dog 3	662
Dog 8	564	Dog 5	700
		Dog 7	681
Average	551	Average	681

Percentage of difference between averages, 22.9

the concentration of hemoglobin is not due to any lack of iron, but to the inability of the body to convert iron into hemoglobin.

IRON ANALYSES OF BONE MARROW

The two femurs of the dog were removed, sawed open, and the marrow removed, ashed and an iron determination made in the usual way on the ash. Table 20 shows the relation between the dogs' weight per kilogram of the dog and the amount of iron found in the marrow removed. Since the amount of iron is very small for simplicity the decimals are treated as whole numbers.

It was not feasible to examine all the bone marrow in the body, but we removed all the bone marrow in the two femurs, which are assumed to be proportional to the size of the dog and to constitute a fair average. It is evident from Table 20 that iron is stored in the bone marrow in the same way as in the liver and spleen.

DOG GROUP 2 (EIGHT DOGS)

Large dogs were bled 25 per cent of the blood volume, the hemoglobin concentration having been determined shortly before. All the dogs were placed on the bread and milk diet. Six of the animals were given 15 c.c. of a 5 per cent solution of iron and ammonium citrate, subcutaneously, every second day. The hemoglobin concentration was redetermined two and a half weeks later, at which time it was much lower in the injected group than in the normal noninjected controls. This suggested a distinctly toxic effect of the iron, so we reduced the dose to one fourth of that previously given, and continued the injections in other respects as before. At the end of one month, the control dogs had fully regained their original hemoglobin concentration, the injected animals being still far below their original figure. The exact figures

TABLE 21—Hemoglobin Concentration in Dog Group 2

	Dog	Before Bleeding, Jan 30	Jan 30 Bled 25 per cent of blood volume	Feb 11	Feb 18	Feb 25
Noninjected controls	1	1.403		1.156	1.228	1.386
	2	1.098		1.016	1.103	1.213
	Average	1.250		1.101	1.165	1.300
Injected dogs	4	1.213		0.997	1.008	1.086
	5	1.034		0.871	1.205	1.003
	6	1.255		0.842	0.948	0.995
	7	1.180		0.778	0.842	0.851
	8	1.274		0.806	0.608	0.917
	9	1.233		0.975	0.937	0.969
	Average	1.199		0.898	0.930	0.955

TABLE 22—Averages in Iron Analyses in Dog Group 2

	Noninjected Controls	Injected Dogs
Livers	26.4 mg per 100 gm of tissue	49.1 mg per 100 gm of tissue
Spleens	147.2 mg per 100 gm of tissue	320.4 mg per 100 gm of tissue

for the controls are 1.25 and 1.3 before bleeding and one month later, respectively. The corresponding figures for the injected animals are 1.199 and 0.955.

In this group of experiments, the dogs showed absolutely no advantage from the subcutaneous use of medicinal iron, it indicates quite clearly that a somewhat larger dose than the usual one is distinctly toxic.

The dogs were killed and their livers and spleens examined.

The relatively large amount of iron in the livers and spleens of the injected group is in striking contrast to the low figures for the hemoglobin concentration in the same group.

DOG GROUP 3 (EIGHT DOGS)

Dog Group 3 was intended to test the value of medicinal iron given intravenously after more or less complete exhaustion of the reserve iron

by repeated bleeding. The animals were kept on bread and milk, that is, an iron-poor diet. We proceeded as follows. The hemoglobin concentration was taken as usual, and then 25 per cent of the calculated blood volume was removed. Ten days later another 25 per cent was removed, and about five weeks later the hemoglobin concentration was again determined (intervening determinations were made, but to save space are not given in Table 22). Then for the third time 25 per cent of the blood volume was removed, and following this three of the dogs were given intravenous injections of from 1 to 2 cc of a 1 per cent solution of the double citrate of iron and ammonium three times a week. Both the injected and the control dogs were kept on the bread and milk diet throughout. The dose of iron given was approximately that given clinically to patients, taking into consideration the relative weights of man and dog. Seventeen and thirty-four days later hemoglobin concentrations were again taken. The results are shown in Table 23.

TABLE 23—*Hemoglobin Concentration in Dog Group 3*

Dog	Before Bleeding March 20	Bled 25 per Cent of Blood Volume March 27	Bled 25 per Cent of Blood Volume April 7	May 13	Bled 25 per Cent of Blood Volume May 16	June 2	June 20
2	1 122	On bread and milk diet	On bread and milk diet	1 292	Bread and milk diet plus intra- venous injec- tion of iron	1 081	1 166
3	1 067			1 206		0 880	0 955
4	1 222			1 122		0 925	1 021
Average	1 137			1 207		0 962	1 047
5	1 145			1 163	Bread and milk diet only	1 032	1 061
6	1 208			1 174		0 961	1 060
1	1 177			1 233		1 063	1 163
Average	1 177			1 190		1 019	1 095
8	0 944			0 864	Placed on partial meat diet	1 078	1 027
10	1 290			1 098		1 081	1 267
Average	1 117			0 981		1 080	1 147

It will be seen that the results are substantially identical. The injected animals had a hemoglobin concentration of 1 137 before the first bleeding, and the last determination showed 1 047. The noninjected controls had 1 177 before the first bleeding, and 1 095 at the last reading. If we prefer to compare the last reading with those taken just before the last removal of blood, May 16 (and this is probably more correct), the figures are essentially the same, except that the injected animals showed a somewhat greater diminution. We have noted the probable significance of this fact elsewhere.

As a further control two of the dogs, after the third bleeding, were placed on a partial meat diet having the same number of calories. The result was as follows. Before the third bleeding, the hemoglobin concentration was 0 981, on the last reading, one month later, 1 147. This shows the manner in which the blood responds to the ingestion of food.

iron, since this group gained nearly 0.17 whereas the other two groups lost 0.16 and 0.10, respectively.

After the last hemoglobin determination, the dogs were killed and the iron content of their livers and spleens determined. The results are given in Table 24.

The much higher content of the livers and spleens in the group receiving iron intravenously is in striking contrast with the failure of the hemoglobin to increase beyond the figures for the noninjected controls. The great increase in iron in the animals receiving a partial meat diet is worthy of note. The last analyses show clearly the difference when food iron is fed.

DOG GROUP 4 (TWELVE DOGS)

In Dog Group 4 the reserve supply of iron in the liver, spleen, etc., was again greatly depleted by repeated bleedings, the animals being on the bread and milk, i. e., non-poor diet. Twenty-five per cent of the calculated blood volume was removed at each bleeding. The average iron-content was first determined on six of the dogs, and found to be

TABLE 24—*Averages in Iron Analyses in Dog Group 3*

	Milk Diet Only (Controls)	Milk Diet Plus Iron Injections	Milk Diet Plus Meat
Livers	87 mg per 100 gm of tissue	278 mg per 100 gm of tissue	531 mg per 100 gm of tissue
Spleens	118.7 mg per 100 gm of tissue	643.1 mg per 100 gm of tissue	461.2 mg per 100 gm of tissue

273 mg per hundred grams of tissue in the liver, and 118.6 gm in the spleen.

Four of the dogs were bled at intervals of about ten days, this being the time required to be sure that the blood volume returns to normal. At the beginning the average hemoglobin concentration was 1.182. One month after the fourth bleeding, the average hemoglobin concentration was 0.749. As 25 per cent of the blood was removed at each bleeding and four bleedings were performed, only 32.2 per cent of hemoglobin should remain in the circulating blood, if none had been thrown in from the reserves in the liver, spleen, etc., or had been taken with the food. It may be safely assumed that in twenty days that elapsed between bleedings the blood volume had been entirely restored, since we showed in Dog Group 1 that the blood volume returns to normal in between seven and ten days. If not, then a *fortiori* the percentage of blood removed was still greater. Now the average concentration before the first bleeding was 1.182 and sixteen days after the fourth bleeding 0.749, that is, 63.5 per cent of the original concentration, so the difference between 32.2 per cent and 63.5 per cent, i. e., 31.3 per cent, had been furnished from the reserve supply if it could not be accounted

for by the iron contained in the bread and milk diet, which is by no means negligible. Moreover, it is possible that when a great strain, as in this case, is placed on the blood forming organs, food iron may be utilized to a greater degree than when such a strain does not exist.

Two additional dogs (Dogs 3 and 4), were treated in precisely the same way, except that after the last bleeding they were given 10 mg of iron and ammonium citrate subcutaneously three times a week. The average hemoglobin concentration in this group before any bleeding was 1.262 and sixteen days after the last bleeding 0.652, i. e., 51 per cent of the original hemoglobin concentration. This is actually 12 per cent less than in the noninjected group.

TABLE 25—*Hemoglobin Concentrations in Dog Group 4*

	Before Bleeding March 31	May 24	June 11	July 8	July 15	Aug 1
Noninjected Controls Dogs 1, 2, 6, 9	1.182	Bled 25%	Bled 25%	Bled 25%	Bled 25%	0.749 i. e., 63.5% of original
Injected Dogs Dogs 3, 4	1.262	Bled 25%	Bled 25%	Bled 25%	Bled 25%	0.652 i. e., 51% of original

TABLE 26—*Averages in Iron Analyses in Dog Group 4*

Noninjected Controls		Injected Group	
Livers		Livers	
Dog 1	31.0 mg per 100 gm of tissue	Dog 3	79.6 mg per 100 gm of tissue
Dog 2	28.1 mg per 100 gm of tissue	Dog 4	83.0 mg per 100 gm of tissue
Dog 6	38.5 mg per 100 gm of tissue		
Dog 9	42.2 mg per 100 gm of tissue	Average	81.3
Average	36.2		
Noninjected Controls		Injected Group	
Spleens		Spleens	
Dog 1	90.1 mg per 100 gm of tissue	Dog 3	340.2 mg per 100 gm of tissue
Dog 2	101.5 mg per 100 gm of tissue	Dog 4	250.8 mg per 100 gm of tissue
Dog 6	111.3 mg per 100 gm of tissue		
Dog 9	123.6 mg per 100 gm of tissue	Average	280.5
Average	107.7		

The dogs were then killed and the analyses of the livers and spleens showed the results given in Table 26. In this group the figures for each dog are given separately, to indicate average variations. In all other groups, to save space, only the averages are given.

It was thus evident that while subcutaneous injections of iron given to animals in which over half the blood had been removed failed to increase the hemoglobin concentration, they did increase the iron content of both liver and spleen more than 100 per cent. In other words, even when there was an urgent need of hemoglobin in the circulating blood iron injections failed to increase it, and while the injected iron was stored up in the liver and spleen it was not capable of being con-

verted into hemoglobin under our experimental conditions. It might be argued that a longer period of injections might have produced an increase in the hemoglobin concentration, but this argument is shown to be invalid by the greatly increased iron content of liver and spleen, this shows that these organs had an abundance of iron to furnish to the blood if only the iron were capable of transformation into hemoglobin. It might be further argued that the entire iron reserve of the liver and spleen, etc., had not been exhausted. This may very well be true, but hemorrhages that remove between 65 and 70 per cent of the blood volume are certainly comparable with very severe anemias, as seen clinically. Further, it seems certain that if iron cannot be converted into hemoglobin, except under a still greater strain on the blood forming organs than is produced by hemorrhages of this magnitude, it would be practically worthless as a remedy.

CONCLUSIONS

1 Inorganic iron, whether given by mouth, subcutaneously or intravenously, is absorbed and may be found especially in the liver and spleen, but is not converted into hemoglobin.

2 Animals made anemic by one or several large bleedings do not recover any more rapidly when inorganic iron is given in any of these ways.

3 The efficiency of food iron is very pronounced, and animals on a diet containing food iron only recover very rapidly from hemorrhages that remove an amount of iron greater than exists in the entire body outside the blood.

4 In the light of the foregoing experiments, the administration of inorganic iron has no therapeutic value in anemia.

VITAL CAPACITY IN A CITIZENS' MILITARY TRAINING CAMP¹

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This study of the vital capacity of a number of students at an army summer training camp, the Citizens' Military Training Camp held at Camp Meade, Md, July, 1924, was planned with a threefold purpose

1 To obtain accurate vital capacity estimations on a relatively large and homogeneous group of individuals The need of further observations as an aid in arriving at some standard by which we might judge all individuals of a given class is certainly great, and the army, with its large and well organized summer training camps, offers a peculiarly good opportunity for studies of value in meeting this need

2 To determine whether or not the improvement in general physical condition resulting from the training undergone is accompanied by a corresponding change (increase) in the vital capacity This general physical improvement is abundantly evidenced by all other criteria at our disposal, and is scarcely subject to doubt from any quarter, and it would not only be of value to learn whether or not the vital capacity increased in keeping with it, but it might even be that the value of vital capacity estimations in general should stand or fall according as it does or does not so increase

3 To determine, if possible, the value of the vital capacity estimation as a criterion of what we call "general physical fitness," as disclosed primarily by a thorough physical examination Some such criterion, while of great importance from the general medical point of view, would have a peculiar and tremendous value for the military services, for the only present means of determining this physical fitness (i e, by thorough physical examination) is not only expensive in money, time and energy, but admittedly allows of many failures and errors And any readily applicable procedure which might, even roughly, separate the "fit" from the "unfit"—which might serve as a sort of yardstick for measuring or classifying the physical manhood of the country—would prove of inestimable military value, especially under the need of a general mobilization, such as arose in 1917

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GENERAL CONDITIONS AND TECHNIC OF STUDY

The subjects of the present study were all young white men, aged from 16 to 20 years, inclusive, and coming from the states of Pennsylvania, Maryland, Virginia and the District of Columbia. They were, in some degree, selected from the average run of young men, for, accompanying their applications for admission to the camp was the report of a physical examination made by a local physician (frequently a member of the Army Reserve), and this report was considered in approving applications. To this extent selection was operative: approved applicants were all presumably in average good health and condition. There was no other factor of selection concerned, the young men came from both urban and rural communities and from various occupations (most of them being students), and they are believed to represent fairly the healthy "normal" youths of their ages.

Since the vital capacity estimations were made as a part of routine physical examinations, the subjects were almost invariably quite nude, in the few cases in which this was not true, there was no clothing that might have interfered with maximal respiratory effort above the waist.

All observations were made on accurately calibrated spirometers of the ordinary water-seal type, quite delicately counterpoised, and mounted on special bases to permit ready transport about the camp. Two such spirometers were used.

No connection or joint had an internal diameter of less than 1 inch except directly at the mouthpiece, where detachable nipples of heavy glass tubing having an internal diameter of $\frac{5}{8}$ inch (1.5 cm.) were used. A large number of these glass nipples were mounted firmly in rubber stoppers, and the whole, being easily removable from the rubber inlet hose, was carefully washed in two changes of water and then in double strength Dobell's solution before being again used. These nipples were of a size readily covered and grasped by the lips, every effort was made to eliminate leakage, and it is believed that in no reading recorded was leakage a factor.

The volume scales on the spirometers were graduated to 20 c.c., and readings to the nearest 10 c.c. were readily made. All readings were made by one or the other of the writers, and great care was used throughout to obtain accuracy of the highest degree.

Each man submitted to test was instructed as to what was desired of him, and was then given three "chances" or "blows," he was urged to make his maximal expiratory effort (following maximal inspiration) at each attempt, and was challenged on the second and third attempts to better his preceding reading. Furthermore, since every observation on every man tested was made in the presence of a number of his fellows, with a resultant spirit or atmosphere of keen rivalry, it is felt

that practically every reading does represent maximal effort at the particular time on the part of the man concerned. All readings were recorded, and the highest of the three efforts accepted as representing the man's maximal expiratory output. The type of expiration required was a sustained single blow, starting more or less gradually, and avoiding all semblance of a sudden or violent blast. It was found that the usual length of the expiratory effort was from about six to seven seconds.

As simple as the actual procedure was, it was found difficult in certain cases to obtain at first what was believed to be a truly maximal expiration, this was due, apparently, to a total lack of knowledge as to how to breathe. In such cases, special effort was exerted to obtain satisfactory results, and, while in most of these the effort was successful, in a few cases it was found impossible to get satisfactory results. The latter cases were not recorded or considered in the findings herein reported.

It was manifestly impossible to make observations on the more than 3,500 men who entered camp in the two days over which the physical examinations extended, but it was felt that, if chance alone governed the selection of subjects, and a sufficiently large group were examined, a truly representative cross-section of the whole would be obtained. Accordingly, the spirometers were set up in a room through which all the men passed on their way from the physical examining hall to the dressing room. There was a constantly passing stream of men, with a constantly available subject at hand. The assistant of the operator of each apparatus, on the completion of an observation on one man, simply "tagged" the man nearest him in the passing line, who became the next subject. This method was followed on the occasion of both the incoming and outgoing examinations. Thus, no element other than pure chance operated to influence the selection of subjects.

COMMENT ON FINDINGS

1 *Normals Before and After Training*—It is to be noted that the two series of observations, as made on 825 healthy young men before, and 443 after, training, are not made on the same men throughout. While there were 103 of these men who chanced to have observations made on them on both occasions, it was impossible to control this feature, and any discussion or conclusions presented herein regarding the effect of the training undergone on vital capacity in the normal are based on the acceptance of the respective groups as fairly representing cross-sections of the total group of young men at the respective times of observation.

Since there were 103 men examined on both occasions, and since the first group of 825 represents approximately 23.5 per cent of the men accepted for the camp and the second group of 443 is slightly more

than 13 per cent of the men who finished the camp, this assumption is not believed to be unfounded. Furthermore, it will be noted on comparing Tables 1 and 2 that the numbers of men in both the total and separate age groups in the former are uniformly approximately double the corresponding figures in the latter chart. This would seem conclusive evidence not only that the selection of these individuals for study was on each occasion directed by chance, but also that both groups are respectively representative of the camp totals before and after training.

TABLE 1—*Data on Eight Hundred and Twenty-Five "Normal" Men Before Beginning Training*

Age, Years	No in Group	Height, Cms	Weight, Kilograms	Body Surface Sq M	Chest, Cm			Average Actual Output, Cc	Average Vital Capacity, Cc Per Sq M Body Surface
					Inspir ation	Expir ation	Expan sion		
16	114	171.2	59.3	1.69	88.1	80.5	7.6	4,128	2,437
17	406	171.2	59.8	1.70	88.4	80.8	7.6	4,104	2,413
18	167	172.7	62.1	1.74	90.7	82.3	8.4	4,397	2,527
19	90	171.7	63.0	1.74	91.9	84.3	7.6	4,360	2,504
20	48	171.0	63.2	1.74	91.4	83.3	8.1	4,299	2,467
Average 825		171.5	60.7	1.71	89.4	81.5	7.9	4,206	2,453

Table 1 shows the physical data gathered on 825 healthy young white men between 16 and 20 years of age, inclusive, at the opening of the training camp. These men were selected by chance (as described above) from a group of more than 3,500 similar young men, and as these data were obtained before beginning training, they represent what we might expect to find in the average young man of this type and these ages.

TABLE 2—*Data on Four Hundred and Forty-Three "Normal" Men After Training*

Age, Years	No in Group	Height, Cm	Weight, Kilograms	Body Surface, Sq M	Chest, Cm			Average Actual Output, Cc	Average Vital Capacity, Cc Per Sq M Body Surface
					Inspir ation	Expir ation	Expan sion		
16	60	170.8	59.1	1.69	88.9	80.8	8.1	4,292	2,533
17	222	171.5	60.1	1.70	89.2	81.3	7.9	4,396	2,577
18	88	172.3	62.8	1.73	91.2	83.3	7.9	4,606	2,641
19	51	172.9	64.5	1.77	92.2	84.1	8.1	4,764	2,694
20	22	173.8	62.3	1.75	91.2	83.1	8.1	4,619	2,636
Average 443		171.9	61.1	1.72	89.9	82.0	7.9	4,477	2,602

Table 2 shows similar data on 443 men similar to the preceding 825, at the close of the four weeks' training period. The conditions surrounding the observations made on the two occasions were identical in every respect except that these 443 men had just completed the prescribed course of training.

In both Tables 1 and 2 an additional column has been added, showing the "vital capacity per square meter of body surface" This calculation is of value (just as in basal metabolism calculations) in making comparisons between individuals or between different observations at different times on the same individual There is no doubt in our minds that the body surface, as determined from the height-weight formula of Du Bois and Du Bois, is the physical characteristic to which the expiratory output can most correctly be referred for comparison, and this is the basis on which the calculations shown in these columns are made

While the height and weight measurements were not made by us personally, these measurements were made under our observation and by individuals fully understanding the need for accuracy, and they are believed to be entirely accurate and reliable On the other hand, it may be remarked that, as a result of our experience in this study, we feel that routine chest measurements are not only unreliable but worthless These measurements were, however, recorded, and are included herewith simply to make the record complete

TABLE 3—*Comparison of Body Surface, Average Actual Output and Vital Capacity in Cubic Centimeters Per Square Meter of Body Surface for "Normal" Men Before and After Training (From Tables 1 and 2)*

Age, Years	Time of Observation	No in Group	Body Surface Sq M	Average Actual Output Cc	Increase		Average Vital Ca pacity, Cc Per Sq M Body Surface	Increase	
					Cc	Per Cent		Cc Per Sq M Body Surface	Per Cent
16	Before	114	1.69	4,128			2,437		
	After	60	1.69	4,292	164	4.0	2,533	96	3.9
17	Before	406	1.70	4,104			2,413		
	After	222	1.70	4,396	292	7.1	2,577	164	6.8
18	Before	167	1.74	4,397			2,527		
	After	88	1.73	4,606	209	4.8	2,641	114	4.5
19	Before	90	1.74	4,360			2,504		
	After	51	1.77	4,764	404	9.3	2,694	190	7.6
20	Before	48	1.74	4,299			2,467		
	After	22	1.75	4,619	320	7.4	2,636	169	6.9
Average	Before	825	1.71	4,206			2,463		
	After	413	1.72	4,477	271	6.4	2,602	139	6.1
Appendix—Similar averages for 103 normal individuals examined both before and after training									
Average	Before	103	1.73	4,308			2,481		
	After	103	1.73	4,524	216	5.0	2,606	125	5.0

Table 3 brings together from Tables 1 and 2, for the several age groups, the body surface, volume output and vital capacity findings for the two observations before and after the training It will be seen from this chart that every age group shows an increase in both actual expiratory output and in the calculated "cubic centimeters per square meter of body surface" The appendix to Table 3 presents the averages of the same data for the 103 men on whom observations were made at both

the beginning and the close of camp. It will be seen from the figures given that the increase in actual volume output as a result of the training amounted to 5 per cent for these 103 men as against the 6.4 per cent for the larger corresponding groups covered in Table 3, while the vital capacity in terms of cubic centimeters per square meter of body surface also increased by 5 per cent for the 103 men as opposed to the 6.1 per cent shown by the larger corresponding groups.

This increase of 5 per cent in both actual volume output and vital capacity for these 103 men is slightly less than the corresponding increases shown by the larger groups of 825 and 443 men, but, accepting the latter figures as being more accurate because of the larger numbers concerned, it is evident that these increases approximate from 5 to 6.4 per cent.

As to whether the amount of this increase is of great significance or not, we cannot say. There is no comparable work reported in the literature, and these findings are presented for what they may be worth and with the hope that others may check them.

The data obtained at Camp Meade have been analyzed in numerous ways, and certain observations may be worth recording at this point. A comparison of Tables 1 and 2 will show that there was no change of any moment as a result of the training in either height, weight, body surface or chest measurements, the increase in actual expiratory output and vital capacity has already been discussed.

The sequence of the volumes of the expiratory efforts was tabulated, and it was found that, of the 825 men examined previous to training, 14.8 per cent made their maximal effort on the first "blow," 23.9 per cent on the second, and 61.3 per cent on the third, while of the 443 men observed at the close of the training period, 14.2 per cent made their maximal effort on the first attempt, 27.1 per cent on the second, and 58.7 per cent on the third. These figures show close agreement for the two groups in this respect, it is evident that the training did not influence the position in the three attempts of the maximal effort, and that, in routine vital capacity estimations on young men, we can expect about 15 per cent to reach their maximal output on the first effort, 25 per cent on the second, and 60 per cent on the third. No relation between the type of individual and this sequence is discernible, and no explanation for these facts is available.

While there are no figures to support the observation, it is very definitely the opinion of both of us that youths of the "long waisted" type gave uniformly (and frequently markedly) higher readings than did those of approximately the same height but shorter chest conformation.

Many of these youths of slender stature and with comparatively small actual chest measurements far surpassed in expiratory volume those with much greater actual chest measurement but of shorter thoracic length

This fact soon became striking while making the actual observations with the spirometers, and it early became evident that, for youths of approximately a given height (disregarding entirely the anteroposterior and lateral diameters of the chest) the expiratory output was at least roughly proportional to the length of the thorax. Whether or not this was due to a difference in potential mobility of the chest walls, or perhaps to the relative degrees of thoracic and abdominal breathing, cannot be said. Neither can we speak with regard to comparative actual lung capacity (as opposed to expiratory output) in these men, but it would seem certain that we can expect from the slender, long waisted type of young man a definitely greater maximal expiratory output than from the youth of approximately the same height but of shorter thoracic length.

2 Group Below Par on Physical Examination—The purpose of this section of the article is to compare with the results on a normal and "physically fit" group of young men, as presented in the preceding section, the findings on another group of similar young men, of corresponding ages and examined in like manner, but found, on physical examination "unfit" for full military duty.

This second group of young men, found to be either generally "below par" or subject to some abnormality, in either case sufficient to prevent their (at least immediately) undertaking full military duty, were assigned together in a "Special Training Company," and their training program, especially for about the first two weeks, differed somewhat from that which was routine for the camp at large. There was no serious organic defect or disease among them, they were separated from the other students because it was felt that they would be unable to stand the training pace set for the rest of the camp, and that they would probably prove something of a hindrance in any regular training company to which they might be assigned. There was the further idea underlying the formation of this company of determining what could be done, by means of a specially devised and carefully regulated and graduated system of training, toward bringing these youths up to the average standard of the camp.

The causes for assignment to this special company of the 115 men studied are listed on the next page. The conditions named are exceedingly abbreviated summaries of the findings reported by the examining medical officers.

Underweight only		87	87
Underweight with	{ Defective vision	5	
	{ Goiter	1	
	{ Varicocele	1	
	{ Flatfeet	1	
	{ Defective teeth	1	
	{ Short leg	1	10
Cardiac conditions	{ Pulmonic murmur, systolic function	2	
	{ Mitral insufficiency, well compensated	1	
	{ Mitral murmur, systolic	1	
	{ Apical murmur, systolic not transmitted	2	
	{ Apical murmur, transmitted from left to right	1	
	{ Apical murmur, systolic, functional	2	
	{ Cardiorespiratory murmur, apical	2	
	{ Rapid heart with apical cardiorespiratory murmur	1	
	{ Rapid, irritable heart	1	13
Flatfeet only		2	2
Spinal curvature, slight		2	2
Unequal pupils, old traumatic		1	1
			115

It will be seen from the foregoing data that ninety-seven (84 per cent) of the 115 men were underweight according to the army's age-height-weight table of requirements, and it may be remarked that, in practically every case, the degree of underweight was such as to be manifest on casual inspection.

This condition was the primary one concerned in the formation of the special company, and it was the controlling factor in the training schedule outlined for the company.

That there was a distinct physical inferiority manifested by the men assigned to this company is definitely borne out by their subsequent records in the so-called "physical efficiency test," which is coming into more or less general use among schools and colleges. This test consists of the bar vault, high jump, broad jump and 100-yard dash, and certain arbitrary values are assigned to each for different ages. Under this test, according to the company commander's report, the Special Training Company's average was 39, while the students in the camp at large averaged 50.5.

With regard to the special handling of these men, they were, in the first place, under the constant and detailed supervision of a medical officer, and the line officers in charge of the company were all selected for their aptitude for the purpose in question. For the first eight days, drills started one-half hour later than for the other students, this time being spent by the men in their barracks, undergoing inspection, or at lectures by the medical officer, etc. After this eight day period, their drills began at the time routine for the camp. Throughout their four weeks of training, they had a half-hour rest period from 9.30 to 10 a. m., and again from 3.30 to 4 p. m., each day, during both of which periods a lunch consisting of 1 pint of milk and graham or similar crackers was served. This lunch was in addition to the regular meals served at the usual mess hours.

Regarding the diet of these men, the company commander, in his report to the commanding general of the camp, says

The diet consisted of the same meals as were provided the other citizens military training camp companies except that coffee, condiments, certain vegetables (such as cucumbers) and pastries were eliminated, and such items as lemonade, cocoa, other fresh vegetables, eggs, custards, light desserts and milk were added. Milk with graham crackers or any other slightly sweet cookie were served at 9 30 a m and 3 30 p m. These lunches were highly appreciated. Cool water was available, and at all times they were urged to drink large quantities. The men were encouraged to take considerable time in which to eat and were never hurried out of the mess hall.

In addition to the foregoing, much attention was paid to general hygiene of the person. As much time as possible was spent in the open, and active play, outdoor calisthenics and games were encouraged and participated in by all members of the company.

A complete study of the vital capacity before, during and on completion of training was made on thirty-eight men of this Special Train-

TABLE 4—Averages for Thirty-Eight Men on Whom Four Observations Were Made, with Actual and Percentage Increases Resulting from the Training Undergone *

Date	Height, Cm	Weight, Kilograms	Body Surface, Sq M	Chest, Cms			Average Actual Output, Cc	Average Vital Capacity, Cc Per Sq M Body Surface
				Inspira- tion	Expira- tion	Expan- sion		
July 1	165 4	49 6	1 53	83 1	75 7	7 4	3,593	2,344
July 10	165 4	49 8	1 54	78 0	70 6	7 4	3 721	2,416
July 18	165 4	50 4	1 54	80 0	71 9	8 1	3 742	2,428
July 24	165 4	50 8	1 55				3,820	2,470
Increase	0	1 2	0 02				227	126
Per cent	0	2 4	1 3				6 3	5 4
Appendix—Same averages for three observations on 115 men of Special Training Company, the last being on completion of the training program. Presented as evidence that data given above for thirty-eight men are truly representative of the 115. There is remarkable agreement between figures for corresponding dates.								
July 10	165 4	50 0	1 54	78 5	71 4	7 1	3,732	2,419
July 18	165 6	50 4	1 54	78 7	71 1	7 9	3 740	2 424
July 24	165 4	51 0	1 55				3,850	2 470

* The first observation was made before beginning, and the last on completion of, the training program.

ing Company. The averages of the findings obtained on the four examinations are presented in Table 4.

The number of men so studied is admittedly small, but three subsequent observations in this "below par" group included 115 men. The results of these three examinations are given in the appendix to Table 4. The latter figures, in their remarkable agreement with those of Table 4 proper for the corresponding examinations on the thirty-eight men are presented as evidence justifying the contention that the figures for the thirty-eight men are not only representative of the larger group of 115, but also should be accorded the weight of the larger number in drawing conclusions as to the effect of the training on them. This the writers have done, and in comparing this special company with the camp at

large, and in drawing conclusions as to the effect of the training on the two groups, the findings on these thirty-eight men have been accepted as being truly representative of the entire special company

It will be seen from a study of the several tables that every recorded observation on the "below par" group (with the possible exception of chest expansion) is definitely lower than the corresponding figures for the "normals "

The differences, before training, for body surface, actual expiratory output and vital capacity in terms of cubic centimeters per square meter of body surface are shown in Table 5 A, while a similar comparison following training is given in B of the same table

From these comparative tabulations, it will be seen that the average actual expiratory output of the Special Training Company was slightly less than 15 per cent below the "normal" at both the beginning and the conclusion of training, while the vital capacity of the special company,

TABLE 5—Comparison of Body Surface, Average Actual Output and Vital Capacity in Cubic Centimeters Per Square Meter of Body Surface, for "Normal" and "Below Par" Groups Before Beginning Training

		Body Surface, Sq M	Average Actual Output, C c A	Difference (Be- low Normal)		Average Vital Capacity, C c Per Sq M Body Surface	Difference (Below Normal)	
				C c	Per Cent		C c Per Sq M Body Surface	Per Cent
"Normals"		1 71	4,206			2,453		
Special Training Company		1 53	3,593	613	14 6	2,344	109	4 4
			B					
Same comparisons, for same groups as above, after training								
"Normals"		1 72	4,477			2,602		
Special Training Company		1 55	3,820	657	14 7	2,470	132	5 1

in terms of cubic centimeters per square meter of body surface, was below that of the "normals," by 4 4 per cent , before, and by 5 1 per cent after, training These facts force the conclusions, first, that while the men of the special company were definitely below the normal in both actual expiratory output and vital capacity both on beginning and at the completion of training, the differences between the two groups were approximately the same for both occasions , and second, that the men of the special company were benefited by the training in roughly the same proportion as were the men in the camp at large

The latter conclusion is borne out by a comparison of Table 4, which also shows the increases as a result of training for the special company, with Table 3 and its appendix, which present the same data for the camp at large For the 103 "normal" individuals examined at both the beginning and the end of training, the increase in actual expiratory output and vital capacity were both 5 per cent , for the large cross-section groups

of normals these increases were respectively 6.4 and 6.1 per cent. The increase for the Special Training Company lies in both cases between the preceding, being respectively 6.3 and 5.4 per cent.

CONCLUSIONS

1 The actual maximal expiratory output of the class of normal white men covered by the study herein presented (aged from 16 to 20 years, inclusive, and coming from the states of Pennsylvania, Maryland, Virginia, and the District of Columbia) ranges from about 4,100 to about 4,400 c c, with an average for the entire group of about 4,200 c c.

2 The vital capacity in terms of cubic centimeters per square meter of body surface for the same group ranges from about 2,400 to about 2,500, with an average of approximately 2,450.

3 The course of training as prescribed in an army summer training camp results for all age groups concerned in an increase in both the maximal expiratory output and the vital capacity in terms of cubic centimeters per square meter of body surface. The increase in each is approximately from 5 to 6 per cent.

4 While the definite significance of this degree of increase in the vital capacity is not certain, the vital capacity does (to the degree shown) increase as the general physical condition of the individual improves.

5 The vital capacity of the young men adjudged "below par" on physical examination was, before training, definitely lower than that of the "normal," by 14.6 per cent for the actual expiratory output, and by 4.4 per cent for the output per square meter of body surface. These differences were practically unchanged by the training undergone, being respectively 14.7 and 5.1 per cent at the end of camp.

6 While the significance of this amount of difference in vital capacity between the "fit" and the "unfit" is yet to be determined, the procedure is, at least to this degree, a measure of general physical fitness.

THE EFFECT OF PHENOBARBITAL (LUMINAL) ON BLOOD PRESSURE IN ARTERIAL HYPERTENSION III

A PRELIMINARY REPORT

C M GRUBER, M D
H H SHACKELFORD, M D
AND
A M ECKLUND, M D
ST LOUIS

The history of drug treatment in arterial hypertension has not been an encouraging one. Some of the commonly employed drugs that have enjoyed a certain degree of popularity at one time or another are tinctures of aconite, veratrum viride and digitalis, potassium iodid and sodium iodid, the nitrites (nitroglycerin, sodium nitrite, erythrol tetra-nitrate), chloral hydrate, and the recently introduced benzyl esters, such as benzyl benzoate, benzyl acetate and benzyl mandelate. It appears therefore, rather an impertinence to report on yet another drug to be eulogized, tested, doubted and perhaps ultimately discarded.

In the outpatient department at Washington University School of Medicine, two patients were referred to us with arterial hypotension, one a girl, aged 19, suffering from epilepsy, the other an elderly man. The former had a systolic pressure of 88 mm of mercury, the latter 104 mm. It was noted that both patients were taking phenobarbital (luminal) in fairly large doses. We then recalled that one of our hypertension patients had suffered from insomnia and had been given phenobarbital, $1\frac{1}{2}$ grains (0.09 gm) three times a day. Looking over his history and record sheet, we found that the week following the use of phenobarbital his systolic blood pressure had fallen from 250 to 190 mm of mercury and his diastolic from 120 to 110 mm. Because of these chance observations phenobarbital was tested as to its value as a blood pressure lowering substance in arterial hypertension cases.

Phenobarbital (luminal) and sodium phenobarbital (luminal sodium) were first used in Germany in 1912 and have since been fairly widely employed both here and abroad in the treatment of epilepsy. As their value as hypnotics in insomnia, due to restlessness, and as sedatives in the treatment of epilepsy are well known, a review of these effects is not to be included in this article.

In 1912 Impens¹ first studied experimentally the pharmacology of phenobarbital and sodium phenobarbital. He determined the hypnotic

* From the Departments of Pharmacology and Internal Medicine (Outpatient Department), Washington University School of Medicine

¹ Impens. Deutsch med Wchnschr 38 945, 1912

and toxic doses of phenobarbital in the cat, dog and rabbit. He found from 25 to 30 mg per kilogram in the cat, 40 to 50 mg per kilogram in the dog and 80 mg per kilogram in the rabbit necessary to produce sleep. This hypnotic dose was found to be one-fourth, one-third and one-half the toxic dose determined for the cat, dog and rabbit, respectively. Much larger doses were found to be necessary with sodium phenobarbital though the proportion to the toxic doses remained the same.

He found phenobarbital and sodium phenobarbital like most other hypnotics to reduce blood pressure. He cites three experiments on cats showing these drugs to produce a decrease in blood pressure. He states in his preface that the stage of reduction is often preceded by a period of increased pressure, "Eine Erhöhung des Blutdruckes vor der Erniedrigung wird Zuerst beobachtet," and cites the following experiment. A 2,520 gm cat had a control pressure of 130-137 mm of mercury before the injection of 0.05 gm per kilogram of sodium phenobarbital (luminal sodium) into the jugular vein. The time and blood pressures following the injection were

3 minutes, 90 mm	5 minutes, 134 mm	9 minutes, 146-153 mm
14 minutes, 145 mm	24 minutes, 151 mm	22 (or 32?) minutes, 148-166 mm
44 minutes, 124-150 mm	71 minutes, 112-140 mm	84 minutes, 114-130 mm

It will be seen that if a rise in blood pressure can be attributed to the drug in this experiment it must have occurred after the fall in blood pressure and not before.

He found the rate of respiration to be decreased but the depth increased by phenobarbital. Large doses produced respiratory paralysis, the heart continuing to beat some time longer. The kidneys were not damaged although part of the drug passed through them unchanged, the remaining portion being destroyed in the tissues. Wetzel² finds it possible to produce deep sleep in experimental animals with phenobarbital, without change in respiration and circulation.

Gruber and Baskett³ noted that sodium phenobarbital and phenobarbital decrease the blood pressure in dogs, cats and rabbits when given intravenously, intraperitoneally or by mouth. They produce acceleration of respiration with decreased depth in small doses and respiratory paralysis with large doses only. The heart was noted to be slowed in rate, decreased in force of contraction and slightly dilated. The cutaneous and kidney blood vessels dilate simultaneously with a constriction of those of the spleen and loop of intestine. Perfusion experiments showed sodium phenobarbital to act peripherally and not centrally. The secretion of urine was not interfered with until the blood pressure became low.

2 Wetzel. *Berl klin Wchnschr* **49** 937, 1912.

3 Gruber and Baskett. *J Pharmacol & Exper Therap* **30** 219, 1925, *J Lab & Clin Med* **10** 630, 1925.

Gregor⁴ tested the side actions of phenobarbital (luminal) on the blood pressure in human beings. In eighty cases tested, he observed a fall in blood pressure with 0.2 gm twelve hours after administration in two instances only. He employed 0.4 gm each in seven women (Table 1).

It will be observed that failure of the drug to produce a fall in blood pressure occurred in one case only and that, the only hypertension case in the series.

Loewe⁵ noted a fall in blood pressure after prolonged use of phenobarbital and considered this a side action of the drug.

Rissmann⁶ and Miltner⁷ report the use of phenobarbital in sixty cases of eclampsia, but give no data as to its value on blood pressure.

TABLE 1—Results of 0.4 Gm of Phenobarbital (Luminal) in Seven Women (Gregor)

Blood Pressure Before	Blood Pressure 12 Hours Later
150 - 92	126 - 84*
124 - 82	104 - 73
142 - 80	130 - 88
184 - 100	188 - 102
124 - 67	114 - 74
144 - 100	114 - 74
140 - 90	130 - 86

* Later two attempts to reduce blood pressure failed.

Miltner does say, however, that it is valuable in this condition in that it lowers blood pressure and acts as a sedative to the respiratory system. He makes no further note as to how it affected the blood pressure in his cases, but states that venesection was resorted to in extreme cases. Since their observations, no one has recorded a fall in blood pressure, although over 2,000 cases have been reported on the use of phenobarbital in the treatment of epilepsy by Kino,⁸ La Rue and Thornburgh,⁹ Goldstein,¹⁰ Dercum,¹¹ Kirk,¹² Raecke,¹³ Austin,¹⁴ Foley,¹⁵ Fuchs,¹⁶ Darling,¹⁷

4 Gregor Therap Monatsh **36** 418, 1912

5 Loewe Deutsch med Wchnschr **42** 947, 1912

6 Rissmann Med Klin **11** 427, 1915, Ztschr f Geburtsh u Gynak **40** 377, 1916, *ibid* **41** 425, 1917

7 Miltner, T Monatschr f Geburtsh u Gynak **53** 137, 1920

8 Kino Therap d Gegenw, September, 1912

9 La Rue, F G, and Thornburgh, H T Kentucky M J **21** 681 (Dec) 1923

10 Goldstein Deutsch med Wchnschr **38** 987, 1912

11 Dercum, F X J Nerv & Ment Dis **1** 168, 1919, Therap Gaz **43** 609 (Sept.) 1919, J Ment Sc, July, 1914

12 Kirk, C C Am J Insan **77** 559 (April) 1921, J Arkansas M Soc **17** 128 (Nov) 1920

13 Raecke Med Klin **8** 865, 1912

14 Austin, M L Ohio State M J **17** 683 (Oct) 1921

15 Foley Institution Quart, Springfield, Ill, June 30, 1921

16 Fuchs Neurol Centralbl **33** 128, 1914

17 Darling, I A Arch Neurol & Psychiat **9** 478 (April) 1923

Galla,¹⁸ Grinker,¹⁹ Watkins,²⁰ Sands,²¹ Small,²² Hughes,²³ Hauptmann,²⁴ and others

METHOD

The method was practically the same as that used in studying the effect of benzyl benzoate on arterial hypertension²⁵ In all instances the patients selected for this investigation had systolic blood pressures over 175 mm of mercury at the beginning of observation Some of the patients were the same ones previously reported on²⁵ In all cases except one, the blood of these patients gave a negative Wassermann reaction Except in the hospital cases, all the blood pressures were taken with the same mercury manometer The maximum pressures were determined by the Koroitkoff sounds²⁶ with a stethoscope The fourth sound was taken as the diastolic pressure

The ambulatory patients were seated near a desk in a quiet room The left arm, from which the blood pressure readings were made, rested comfortably on the desk Blood pressure readings were taken within two minutes after the patients had sat down and thereafter at ten minute intervals for twenty or more minutes These pressures were then averaged to give the figures presented in Table 2 In all the observations the subjects were requested to sit as quietly as possible and not even to speak to the observer In the hospital cases, the patients were kept in bed and the pressure taken while the patient was in the recumbent position As nearly as possible the blood pressure readings were taken at the same hour each day These were recorded for several days or even weeks until the control blood pressure level was established before the drug was prescribed In the early observations readings were taken either every day or every other day, but later they were taken at weekly intervals As nearly as possible, the patients were kept on a prescribed low protein diet

All the ambulatory patients received 15 drops of a saturated aqueous solution of potassium iodid three times a day Some of the patients received in addition 10 or 15 (depending on the size of patient) drops of tincture of digitalis three times a day As long as the blood pressure was falling under this treatment and diet, phenobarbital was withheld

18 Galla Brit M J 2 320, 1921

19 Grinker, J Experiences with Luminal in Epilepsy, J A M A 75 588 (Aug 28) 1920, Phenobarbital in Epilepsy, *ibid* 79 788 (Sept 2) 1922

20 Watkins, H M New York M J 112 891 (Dec 4) 1920

21 Sands, I J Arch Neurol & Psychiat 5 305 (March) 1921

22 Small, V R Virginia M Monthly 48 386 (Oct) 1921

23 Hughes, W N Rhode Island M J 4 157 (Dec) 1921

24 Hauptmann Munchen med Wchnschr 59 1907, 1912

25 Gruber, C M, and Shackelford, H H J Lab & Clin Med 9 685 (July) 1924

26 Erlanger, J Am J Physiol 40 86 (March) 1916, *ibid* 55 9 (Jan) 1921

TABLE 2—Summary of Laboratory Findings in Twenty-Seven Ambulatory Patients¹

Case	Laboratory Findings										Diagnosis and Remarks Concerning Toxæ Condition During Treatment		
	Blood					Urine							
	Nonprotein Nitrogen in Mg per 100 Cc Blood in 2 Hrs	Percent Phenol	Albumin	Specific Gravity	Urea	Blood Pressure After Routine Treatment and Diet	Blood Pressure During Phenobarbital Treatment	Blood Pressure After Phenobarbital Treatment					
1 White woman, aged 61	34.5	52	0	1.019	Hyaline	193-101	No Days 20	Blood Pressure 163-94	No Days 4	Blood Pressure 139-82	No Days 9	Blood Pressure 164-90	Arteriosclerosis, chronic infectious arthritis Drowsy
2 Colored woman, aged 68	24 33.4 33.3	30 39 36 42	+	1.004 to 1.015	Hyaline	230-121	20	219-115	6	171-103	7	215-110	Chronic interstitial nephritis, cardiac decompensation, chronic myocarditis, pruritus
3 White woman, aged 70	29 27.8 30.6	47 45 7	Trace 3+	1.005 to 1.013	Hyaline, granular	224-94	78	231-98 232-106 230-104 249-109 223-101 230-112 243-108	5 4 7 3 2 16	175-78 198-84 185-95 173-103 170-95 176-100 172-98 193-97	15 44 27 21 37	233-106 230-104 249-109 239-112 243-108	Arteriosclerosis, chronic myocarditis, extrasystoles "Uncertain feeling"
4 Colored man, aged 50	62.5	15	3+	1.011	Hyaline, granular	208-132	13	215-130	8	214-131	28	224-133	Chronic interstitial nephritis uremia, duodenal ulcer, pyorrhea
5 White man,† aged 58	34.3 33.9 25.0 30.3 33.3 37.2 31.2	42 54 53 52 41 30 45 36 32.7	+	1.009 to 1.026	Hyaline, granular	230-116	147	250-120 246-116 211-114 203-111 210-113 212-110 191-109 229-123	7 13 2 12 11 34 1	190-90 182-69 173-103 172-103 170-95 176-100 172-98 193-97	28 14 11 20 38 14 14	216-116 211-114 203-111 210-113 211-110 191-109 229-123	Chronic interstitial nephritis, pulsus alternans, arteriosclerosis old left hemiplegia "Gait unsteady at times", less nocturia
6 White woman, aged 53	32.8 27.7	43 30 37 36	+		Hyaline, granular	280- 240-120	11	265-119 231-112	6 1	205-93 185-86	12 18	232-100 227-95	Polynephritis (diabetic), cardiac decompensation, pulsus alternans, hypertension (1916), 240-126, diabetes mellitus, arteriosclerosis
7 White woman,† aged 43	27	42 58	Very faint trace	1.017	Hyaline	176-120	88	161-108	11	139-95	7	143-101	Chronic interstitial nephritis, old right hemiplegia
8 White woman,† aged 48	25.6 23.3	39 30	Very faint trace	1.015	0	200-120	205	188-101 189-107	11 14	154-89 174-97	17 24	177-104 199-102	Mitral regurgitation, chronic myocarditis, chronic interstitial nephritis, hypertension since 1918, 200-120
9 Colored man,† aged 44	27.5 26.6 31.0	39 27 37	+	1.017	0	190-116	93	152-108	3	136-91	13	150-103	Pulmonary tuberculosis (arrested), chronic interstitial nephritis
10 Colored woman, aged 50	37.5 37.7 35	57 45 35	2+	1.006 to 1.008	0	228-110	63	224-109	25	181-100	14	213-120	Chronic interstitial nephritis, old right hemiplegia Vertigo, sensation of filling backward
11 Colored woman, Aged 44	41.7 25.9	42	Trace	1.010	Hyaline	240-125	112	237-118	5	209-118	23	243-127	Chronic interstitial nephritis, old left hemiplegia, arteriosclerosis
12 White man,† aged 61	40 36 40.8	37 40 42	Very faint trace	1.015	Hyaline	207-105	68	207-105 191-104	4 3	169-98 166-97	6 10	194-104 205-112	Chronic interstitial nephritis in general hemiplegia tendency to fall back

13	13 5	65	0	1 025	0	217 110	57	211 112	20	187 108	17	215 108	Pulmonary tuberculosis (irrested), chronic interstitial nephritis
White woman,† aged 42	40 8	35											
14	35 1	27											
Colored man, aged 47	38	65	Very faint trace	1 011 to 1 025	Hyaline, granular	170-121	60	172 126	5	115-101	55	177 121	Arteriosclerosis, old right hemiplegia, chronic interstitial nephritis, chronic prostatitis Verfigo, pricking sensation of skin
15	41 7	61											
Colored woman,† aged 27	33 7	73	+	1 001 to 1 017	Hyaline	250 155	78	260-177	10	276 168	2	266 168	Nontoxic goiter, chronic interstitial nephritis
16	25 7	59											
Colored woman,† aged 47	32 5	48											
17	34 4	46	+	1 008 to 1 019	0	217 127	85	201 125	0	186 111	9	219 125	Arteriosclerosis, chronic myocarditis
18	35 7	50											
Colored woman,† aged 63	35	48	Trace	1 015 to 1 025	0	201 104	35	170-78	1	137 76	12	165 81	Arteriosclerosis, chronic myocarditis
19	28 1	50											
White woman, aged 66	26 6	51	+	1 016 to 1 030	0	201 104	16	204 101	11	165 91			Lupus erythematosus, chronic interstitial nephritis, constipation, pyorrhea Drowsy
20	32	51	Trace	1 010	0	228-110	13	201 121	2	185-95	22	215 127	Chronic interstitial nephritis Drowsy
21	29 4	38	16 (1 hr)					218-121	7	182 118	29	213 127	
22	32 8	39						218 118	14	183 108	1	215 119	
23	31 4	29						218 118	5	191 106			
White man, aged 54	54	24	3+	1 011 to 1 015	Hyaline granular	195 152	5	195-133	9	192-122	14	221 138	Chronic interstitial nephritis, uremia (chronic), myocarditis (chronic), aortitis, cardiac decompensation
24	60 9	17						221 138	7	196-121	7		
25	25	41	0	1 028	Hyaline	190 110	83	141 80	3	127 73	18	183 85	Arteriosclerosis, Parkinson's disease, pyorrhea Drowsy
26	31 5	68				186-89	6	170-89	6	161 70	13	176-76	Arteriosclerosis Drowsy
27	27 8	39	2+	1 010 to 1 021	Hyaline, granular	200 116	21	191 105	21	169 98			Chronic interstitial nephritis, chronic myocarditis, arteriosclerosis, ichthyosis Drowsy, tendency to fall back
28	33 1	29	3+	1 008 to 1 012	Hyaline granular	250 160	16	206-118	5	168-105	7	191 116	Pulsus alternans, chronic interstitial nephritis, cardiac decompensation, syphilis, late Wassermann reaction +
29	33 1	41	0		0	199 90	63	192-89	7	169 77	12	177 82	Chronic gastritis (hyperchlorhydria) Drowsy
30	20 8	44	+	1 015	0	230-130	7	218 126	7	177 103	10	187-109	Chronic interstitial nephritis
31	23 3	65	Very faint trace	1 018	0	217 129	100	250 110	11	175-90	11	207 117	Arteriosclerosis, chronic prostatitis

* The blood pressure readings are given in millimeters of mercury. In all cases the average of the three ten minute blood pressure readings is given. The readings given in the column under routine treatment are those taken the day phenobarbital was administered. All the patients were ambulatory and were treated first with diet and with a saturated aqueous solution of potassium iodid fifteen drops three times a day. Some were given tincture of digitalis, from 10 to 15 drops three times a day, depending on the size of the patient and the kidney involvement. Unless otherwise indicated, phenobarbital was given in 15 grain (0.69 gm) doses three times a day. All patients gave a negative blood Wassermann reaction except Case 21. † This and all following cases so indicated were reported on previously. ‡ The case numbers are the same in each. In this and the following cases so indicated † = 0.75 grain (0.04 gm) given three times a day, § = 1.5 grains (0.69 gm) twice a day, morning and evening # = 3 grains (0.19 gm) three times a day and ° = 1.5 grains (0.69 gm) four times a day

Phenobarbital was not prescribed in larger doses than $1\frac{1}{2}$ grains (0.09 gm) three times a day (In one case the patient took by mistake without ill effects 3 grains [0.19 gm] three times a day for four days) Smaller doses were given to some patients and to some $1\frac{1}{2}$ grains (0.09 gm) were given less frequently

RESULTS

Here, as in our previous article,²⁵ temporary rest alone caused a marked fall in blood pressure In some patients the preliminary treatment of potassium iodid and digitalis markedly lowered the blood pressure

TABLE 3—*Effect of Phenobarbital on Blood Pressure in Patients with Arterial Hypertension at Rest in Bed**

Case	Blood Pressure on Admission to Hospital	Number of Days Rest in Bed	Blood Pressure After Rest in Bed	Number of Days Phenobarbital Was Given	Number of $1\frac{1}{2}$ Grain (0.09 gm) Doses a Day	Blood Pressure During Phenobarbital Treatment	Blood Pressure After Phenobarbital Was Discontinued	Diagnosis and Remarks
1 Age 46	273-128	9	246-134	3	3	208-128	224-112	Myocarditis chronic mitral insufficiency, caries of teeth, general arteriosclerosis, glaucoma chronic interstitial nephritis, hypertrophy of heart
2 Age 39	236-142	6	240-140	3	3	196-116	220-128	Chronic interstitial nephritis, optic neuritis caries of teeth, general arteriosclerosis, metastatic carcinoma of calvarium (osteoplastic), chronic tonsillitis, sphenoidal sinusitis
3 Age 57	260-148	3	248-135	10	3	175-105		
4 Age 44	210-125	7	205-128 190-120	7	2 2	168-100 170-100	190-120	Mitral regurgitation, cardiac hypertrophy, cardiac decompensation, chronic interstitial nephritis, erysipelas The patient died suddenly
5 Age 64	220-120	3	210-115	4	3	180-105	190-110	Arteriosclerosis, general, chronic constipation
6 Age 48	220-108	2	209-104	10	3	160-90		Mitral regurgitation, arteriosclerosis
7 Age 60	240-150	8	230-145	21	3	175-120		Arteriosclerosis, chronic infectious arthritis
8 Age 54	210-148	4	204-140	4 6	3 1	170-120 160-110		Arteriosclerosis, chronic interstitial nephritis

* The blood Wassermann reaction was negative in all cases All the patients were white women varying in age from 39 to 64 years

The immediate results with phenobarbital on arterial hypertension were satisfactory We have used this drug in the treatment of hypertension in twenty-seven ambulatory and eight hospital cases (Tables 2 and 3) In some instances use has been almost continuous for as long as eight months with beneficial results In others the phenobarbital ceased to have an effect after three or four weeks' administration In the ambulatory patients thus treated, a definite fall in arterial blood pressure was noted in twenty-three of the twenty-seven cases In some

of these the drug was tested several times with the same results. In the four instances in which it was not effective, Cases 4, 15, 20 and 22, two of the patients appeared to have severe nephritis with cardiac decompensation. The blood nonprotein nitrogen was high and the urine contained large amounts of albumin. Cases 4 and 20 showed passive congestion of the lungs and liver, edema of the extremities, and no doubt there was a similar involvement of the kidneys. In addition, Case 4 was very obstinate and some doubt has arisen as to whether or not the patient had followed any part of the treatment outlined. Both

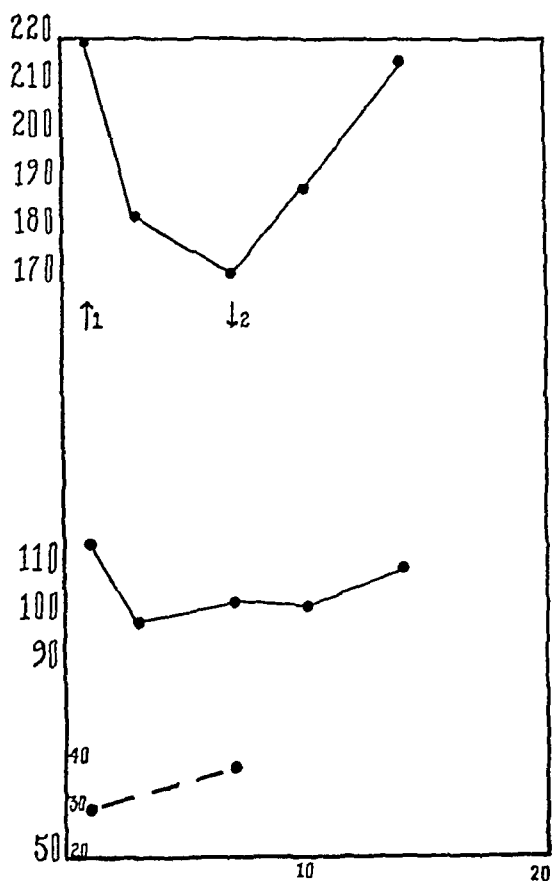


Fig 1

Fig 1—Curve plotted from the data obtained from Case 2, Table 2, a colored woman, aged 68. In this and the following figures, the blood pressure in millimeters of mercury and the percentage of phenolsulphonephthalein excretion for two hours are plotted on the ordinate, the former figures are to the left of the line, the latter to the right, and the time in days on the abscissa. The top record is the systolic blood pressure, the middle the diastolic, and the bottom record the phenolsulphonephthalein excretion. In all cases the average of the three (ten minute intervals) readings is plotted. 1, blood pressure on the day before phenobarbital, $1\frac{1}{2}$ grains (0.09 gm) three times a day was administered, 2, phenobarbital discontinued.

patients were recommended for hospital treatment, which Patient 4 declined. The third case was that of a colored woman, aged 27, Case 15. She had been treated in every way available in the dispensary

without beneficial results In this case, nitroglycerin, $\frac{1}{50}$ grain, caused a rise in blood pressure, sodium nitrite, benzyl benzoate, salt restriction etc, had no effect

In the twenty-three cases in which phenobarbital was effective, the fall in systolic pressure varied during the first series of administrations from 14 to 60 mm of mercury, with an average of 35 mm The fall in diastolic pressure varied from 0 to 30 mm of mercury, with an average of 13 mm (Table 2 and Charts 1 and 2) In order to conserve space in Table 2, the average readings are given, i e, the average of the three readings taken on a single day Although many blood pressure readings were made while the patient was under our observation and

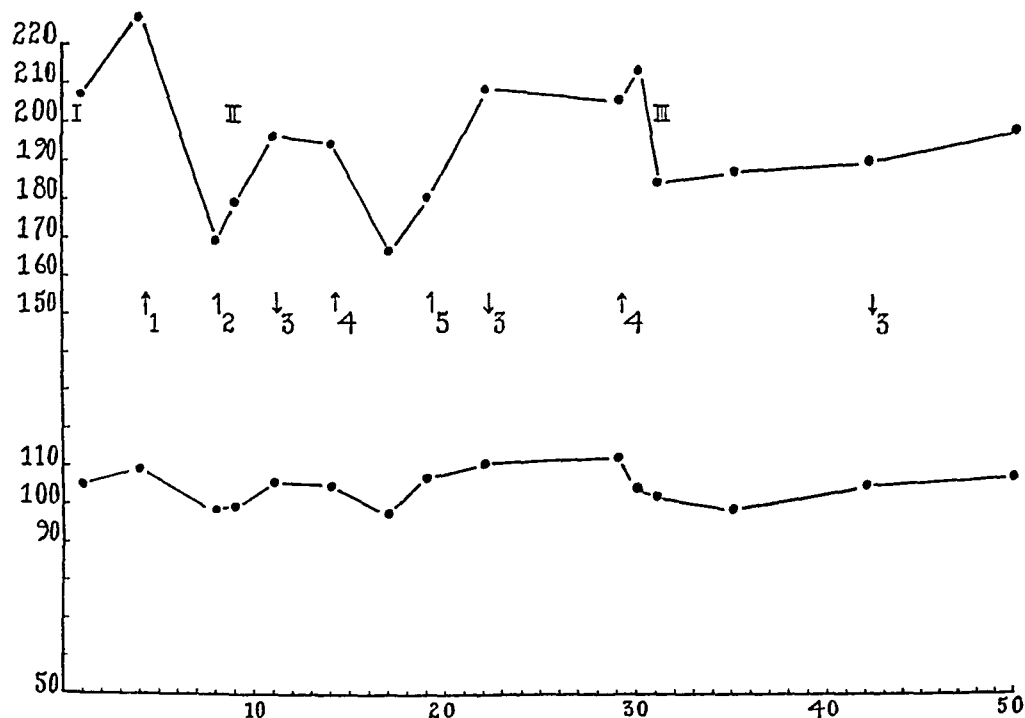


Fig 2

Fig 2—Curve of Case 12, Table 2, a white man, aged 61 1, phenobarbital 3 grains (0.19 gm) three times a day, 2, $\frac{3}{4}$ grain (0.04 gm) three times a day, 3, phenobarbital discontinued, 4, $1\frac{1}{2}$ grains (0.09 gm) three times a day, 5, $\frac{3}{4}$ grain (0.04 gm) once a day, I, nonprotein nitrogen, 40 mg per hundred millimeters of blood and phenolsulphonaphthalein, 37 per cent, II, nonprotein nitrogen, 36.3 mg and phenolsulphonaphthalein, 40 per cent, III, nonprotein nitrogen, 40.8 mg, and phenolsulphonaphthalein, 42 per cent

treatment, only the blood pressure on the day immediately before treatment and the lowest pressure during treatment and again the highest pressure following treatment are given here We have thus far found no indication that phenobarbital increases blood pressure before producing its depression as was thought to be the case by Impens¹

Charts 1 and 2 are presented to show more clearly the effect of phenobarbital on both systolic and diastolic blood pressures. In Chart 1, a colored woman, aged 68, was treated in the routine manner in the dispensary for several weeks before receiving phenobarbital. Her average blood pressure determined in millimeters of mercury on entrance was 230 systolic and 121 diastolic. This fell to 219 systolic and 115 diastolic after twenty days of routine treatment. Phenobarbital, $1\frac{1}{2}$ grains (0.09 gm), three times a day was given at 1. Two days later the average blood pressure for the three ten minute periods was 184 systolic and 98 diastolic, and four days later, or six days after beginning treatment, it was 171 systolic and 102 diastolic. Phenobarbital treatment was then withdrawn and the systolic pressure returned to 215 mm of mercury and the diastolic to 110.

In Chart 2, a white man, aged 61, who had previously submitted to a twelve day course of benzyl benzoate treatment without results,²⁷ was given phenobarbital. Through some misunderstanding, this patient took 3 grains (0.19 gm) of phenobarbital three times a day for four days at 1. His average systolic blood pressure for the three readings for the day fell from 227 to 169 mm of mercury and his diastolic from 109 to 98. At 4, the patient took $1\frac{1}{2}$ grains (0.09 gm) three times a day with good results, as seen in the chart. Smaller doses were less effective in lowering blood pressure at 2 and 5. In the chart, each 3 indicates a discontinuance of phenobarbital administration.

HOSPITAL CASES

It may be thought that the fall in blood pressure in the ambulatory cases under phenobarbital treatment was due to the hypnotic action of the drug and consequent quieting of the patient. Such, however, is not the case. Phenobarbital produces the same effect in patients at rest as in those up and about. This mode of therapy was tested in eight hospital cases. The first two cases in Table 3 were cases in Barnes Hospital, while the others were patients at St. Louis City Hospital. The fall in blood pressure in these cases was as marked as in our ambulatory cases.

The results obtained from Patient 1 are plotted in Chart 3. In Chart 3, the patient had been kept in bed for six days before phenobarbital was started at 1. She received $1\frac{1}{2}$ grains (0.09 gm) of phenobarbital three times a day at 1, at 2 the same quantity twice, and at 3 once a day. At 4 the treatment was discontinued. It will be seen that the average of the six readings of the systolic pressure before treatment and the average of all the readings following it show a fall

27 Gruber and Schakelford (Footnote 25, Fig. 4)

of 36 mm, and that the average diastolic pressure fell 15 mm of mercury. The maximum systolic fall was 54 mm and the maximum diastolic fall was 34 mm of mercury.

EFFECT OF PHENOBARBITAL ON KIDNEY FUNCTION

Although phenobarbital is eliminated in the urine, most observers agree that it has no detrimental effect on the kidneys. Among these

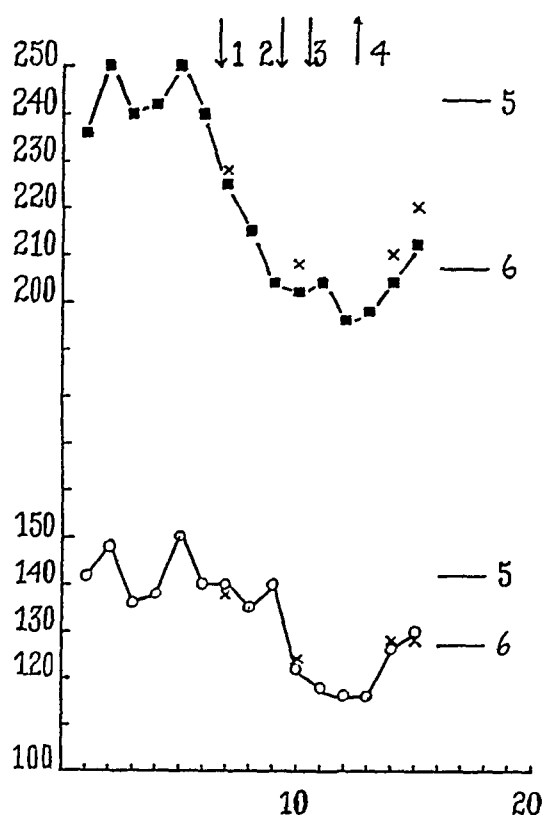


Fig 3

Fig 3—Curve of Case 2, Table 2, a white woman, aged 39. The top record is the systolic blood pressure, the bottom record diastolic blood pressure. The time in days is plotted on the abscissa and the blood pressure in millimeters of mercury on the ordinate. 1, phenobarbital given, $1\frac{1}{2}$ grains (0.09 gm) three times a day, 2, phenobarbital, $1\frac{1}{2}$ grains twice a day, 3, phenobarbital, $1\frac{1}{2}$ grains once a day, 4, phenobarbital discontinued, 5, average of all the blood pressure readings after phenobarbital.

are Impens,¹ Kino,⁸ Kirk,¹² Weber,²⁸ Stein²⁹ and Geisler³⁰. Goldstein,¹⁰ Hueber³¹ and Phillips³² report the appearance of albumin in the urine,

28 Weber, Hannes. *Therap Halbmonatsch* **35** 467 (Aug 1) 1921, *Klin Wchnschr* **1** 998 (May 13) 1922.

29 Stein, J. *Therap Halbmonatsch* **34** 387 (July 15) 1920.

30 Geisler. *Munchen med Wchnschr* **59** 922, 1912.

31 Hueber. *Munchen med Wchnschr* **66** 1090, 1919.

32 Phillips, John. Phenobarbital (Luminal) Poisoning, *J A M A* **78** 1199 (April 22) 1922.

and the latter author believes it also capable of producing nephritis. Our observations in the main support the former view, but certain changes in the phenolsulphonephthalein excretion were noted in our patients while under phenobarbital treatment. In most instances shortly after starting treatment, the percentage of phenolsulphonephthalein excretion increased. This is observed in Charts 1 and 2 and in Table 2. We might be inclined to believe this to be due to an increased kidney function as a result of the fall in blood pressure except for the fact that an increase occurred in some cases in which the blood pressure was already fairly low. On the other hand, prolonged administration seems to decrease the rate of elimination of phenolsulphonephthalein (Table 2). This may be explained as being due to increased rate of destruction of the phenol derivatives after the tissues have been exposed to phenobarbital for a long time. After the withdrawal of phenobarbital for a short time the phenolsulphonephthalein excretion appears to be restored to its former level. These results do not attempt to prove anything but are simply suggestive of changes since wide variations from week to week occur, even in normal subjects.

The effect of phenobarbital on the kidney function was studied on two female dogs. These animals weighed 20 kg each, and were given phenobarbital by mouth. The phenolsulphonephthalein readings were taken before and during administration. The urine was collected by catheterization. One animal received phenobarbital, $1\frac{1}{2}$ grains (0.09 gm) three times a day for fifteen days, after which time she was given 3 grains (0.19 gm) three times a day for nine days. The other was started at once on 3 grains (0.19 gm) three times a day, and was given the drug for nine days. The results were quite uniform. The percentage of phenolsulphonephthalein excreted in Dog 1 before the drug was given was 58 and 54, and during the drug administration 49, 65, 42 and 50 per cent. In Dog 2 the control readings were 44 and 47 per cent, and those while the animal was taking the drug were 48 and 47 per cent. Phenobarbital as far as we could determine has no effect on the kidney function when given in even as large doses as 9 grains (0.6 gm) per day. The urine showed no casts or albumin. It gave a definite sugar reduction with Benedict's and Fehling's solutions on prolonged boiling. This may be due to the elimination of phenobarbital in combination with glycuronic acid. It seems, therefore, that the results noted in our patients must have been due to other factors than the administration of phenobarbital.

TOXICITY OF PHENOBARBITAL

Many men who have used phenobarbital in the treatment of epilepsy believe that the use of phenobarbital without discrimination will be

followed by lamentable results. It is not atoxic. Prolonged use in small doses or its use in large doses over a shorter period may be accompanied by a variety of toxic symptoms. This is especially true in hyper-susceptible persons. There have appeared in the literature over 100 cases of poisoning with four fatalities attributed to its use. Any or all of the following symptoms may be noted after prolonged use of fairly large doses: asthenia, ataxia, vertigo with a tendency to fall backward, either dilatation or constriction of the pupils, conjunctivitis, nystagmus, diplopia, blindness, sleepiness to complete loss of consciousness, headache, mental apathy, hallucinations (auditory and visual), delusions and mental defects (tendency to cry), also mental symptoms simulating alcoholic intoxication (the patient becoming loquacious, excited, exalted and manifesting a flippant behavior), slow slurring speech to total loss, inflammation of pharynx, swollen reddened tongue, with denuded epithelial areas, which takes on a raw, beefy appearance, red gum line, appearance of thin walled blisters filled with blood fluid on the lips, which later ulcerate, anorexia, nausea, vomiting, diarrhea with mucous stools containing blood, atony of the intestine, atony of the urinary bladder, either anesthesia of the skin or pruritis, cutaneous rash resembling measles or scarlet fever, with small petechiae, increased body temperature, Cheyne-Stokes respiration, either bradycardia or tachycardia, and fall in blood pressure.

Although a host of changes are seen in toxic cases, yet the drug is relatively nontoxic when given properly. The range between the therapeutic and the toxic dose is large. Patients have taken as much as 3 gm of phenobarbital in a single dose without causing death,³³ and the four deaths attributed to phenobarbital were probably not wholly due to the drug but due to other causes. The most common symptoms are sleepiness, asthenia, vertigo, ataxia and pruritis. In the literature the cutaneous rashes were reported thirty-four times in 109 toxic cases by Gregor,⁴ Galla,¹⁸ Grinker,¹⁹ Weber,²⁸ Hueber,³¹ Phillips,³² Patschke,³⁴ Emanuel,³⁵ Greenwald,³⁶ Haug,³⁷ Blichert,³⁸ Eder,³⁹ Herrmann,⁴⁰ Ashmore,⁴¹ von Klebelsberg,⁴² Zimmermann,⁴³ Strauss,⁴⁴ Chargin⁴⁵ and

33 Nicolai. *Klin Wchnschr* **2** 1891, 1923

34 Patschke. *Neurol Centralbl* **31** 899, 1912

35 Emanuel. *Neurol Centralbl* **31** 563, 1912

36 Greenwald, J G. *New York M J* **116** 356 (Sept 20) 1922

37 Haug. *Munchen med Wchnschr* **66** 1494, 1919

38 Blichert. *Ugesk f Laeger, Kjøbenh* **75** 1149, 1913

39 Eder. *Therap d Gegenw*, Berlin, **53** 258, 1912

40 Herrmann, R. *Klin Wchnschr* **2** 212 (Jan 29) 1923

41 Ashmore, B L. *Boston M & S J* **187** 950 (Dec 28) 1922

42 Von Klebelsberg. *Psychol Neurol Wchnschr*, 1912, p 415

43 Zimmermann. *Therap Halbmonatsch* **34** 79, 1920

44 Strauss. *Therap Halbmonatsch* **31** 338, 1917

45 Chargin, L. *Transactions New York Academy of Medicine, Arch Dermat & Syph* **6** 222 (Aug) 1922

others This rash is usually described as resembling measles or scarlet fever Rosen⁴⁶ enumerates four types of phenobarbital eruptions of the mucous membranes the pemphigoid, morbilliform, scarlatiniform and erythema multiforme Chargin⁴⁵ believes ethylphenylbarbituric acid eruption to be due to the amido or the phenyl radical which presumably breaks up certain of the proteins in the system causing them to act much like foreign proteins The fact that phenobarbital produces cutaneous vasodilatation³ in this area may be an important factor in the production of the rash The drug may produce here a capillary paralysis as well as a relaxation of the arterioles and venules

The four possible fatal cases reported in the literature as a result of phenobarbital administration are those by Schaefer,⁴⁷ Hueber,³¹ Rosenberger⁴⁸ and Ashmore⁴¹ Schaefer administered to a male patient with chronic bronchitis and marked arteriosclerosis 0.4 gm the first night and 0.5 gm subcutaneously the second evening Five days later, the patient developed hypostatic pneumonia and heart failure from which he died The case reported by Hueber was that of a man, aged 40 He was given about 0.5 gm of sodium phenobarbital a day Thirty days later, he developed a rash, anuria, fever, cough, vomiting and active tuberculosis His death was due to the development of active fulminant miliary tuberculosis Hueber doubts very much if death was due to the drug but believes that it may have been a contributing factor Rosenberger's case was that of a woman, aged 31, who took forty 1 grain tablets of phenobarbital and 10 c c of tincture of opium for suicidal purposes She died two days later Ashmore reports a case of a negro man, aged 36, who had taken phenobarbital for forty-one days, after which an eruption appeared on the face and body but disappeared when phenobarbital was withdrawn Without further treatment, fourteen days following the first appearance the eruptions reappeared This was accompanied by edema of the eyelids, conjunctivitis, and reddened membranes of the mouth and throat The tonsils and tongue were swollen, and he presented symptoms of acute bronchitis The temperature did not go above 102.6 F, the pulse rate above 124, or respiration rate above 30 The spots that were separated became more noticeable and confluent and took on the form of a desquamating eczema, with a tendency to ulcerations with pustule formation The patient died twenty-seven days after the first appearance of the symptoms

In our cases (Table 2) we noted no rashes Some of the patients complained of intoxication with a tendency to fall backward, sleepiness,

46 Rosen Transactions New York Academy of Medicine, Arch Dermat & Syph 6 223 (Aug) 1922

47 Schaefer Berl klin Wchnschr 49 1038, 1912

48 Rosenberger Med Klin 15 1150, 1919

vertigo, pruritis, a pricking sensation of the skin and asthenia. None of these symptoms were sufficiently severe to require reduction of the dose to less than $1\frac{1}{2}$ grains (0.09 gm) twice a day.

COMMENT

Phenobarbital (luminal) caused a fall in arterial blood pressure in the treatment of arterial hypertension in about 85 per cent of our cases. On prolonged use it became less and less effective. There were observed no serious side actions. It is, as far as we know, nonhabit forming, according to Dercum,¹¹ Kirk,¹² Raecke,¹³ Darling,¹⁷ Grinker,¹⁹ Hauptmann,²⁴ Bruhl⁴⁹ and Wender and Sampson.⁵⁰ In our own cases, it was withdrawn without the slightest psychic effect on the patients. After withdrawal we did not note in any instance a marked increase in blood pressure above the control level and in many cases it remained low. It seems to have less effect on the increased blood pressure in cases of acute nephritis with cardiac decompensation than on the chronic nephritic cases and on hypertension observed in arteriosclerotic ones. There appears to be some cumulative action, and we believe that the drug should be discontinued for a short time and the patient treated either in a routine manner or with sodium nitrite during these intervals. Prolonged use appears to decrease phenolsulphonephthalein elimination in the urine after an interval of increased elimination. The mode of action has not been determined.

Most of our patients said they felt improved, were temporarily at least stronger and had less headache until the toxic effect was reached. The authors warn against a too sanguine view of phenobarbital in the treatment of hypertension. It is at best not a cure, although the improvement in many instances appears most gratifying. We have cited absolute failure in four cases.

SUMMARY

1 Phenobarbital decreased the arterial blood pressure in 85 per cent of our cases of hypertension in which it was used.

2 It appears to become less and less effective in most instances on prolonged administration.

3 It has the same effect on both ambulatory and hospital patients.

4 In moderate doses it has probably no harmful effect on the kidney tissues and on the phenolsulphonephthalein excretion. Immediately with the fall in blood pressure the phenolsulphonephthalein excretion is increased but later, in some instances, its elimination is

49 Bruhl, F. *Munchen med Wchnschr* 67 990 (Aug 20) 1920.

50 Wender, L., and Sampson, D. G. *New York M J* 116 336 (Sept 20) 1922.

diminished The elimination again returns to its original level after withdrawal of the drug

5 It produces marked toxic symptoms in large doses and therefore should not be prescribed indiscriminately

BASAL METABOLISM AS AFFECTED BY ATMOSPHERIC CONDITIONS

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The constancy in the basal metabolism of human beings has been emphasized recently by Lusk and DuBois,¹ who compiled data showing that a man between 30 and 40 years of age may show a basal metabolism during eleven years within a variation of ± 7.6 per cent. Zuntz² likewise recorded his basal metabolism at intervals over a period of twenty-nine years with little variation. Other physiologic measurements such as body temperature, pulse rate and, in lesser degree, blood pressure under favorable conditions show a similar uniformity.

A high environmental temperature is an important factor in altering this uniformity in physiologic reactions, and often in certain industries marked deviations from the normal occur in workmen who are daily exposed to abnormally high temperatures.

While the human body has the ability to adapt itself to a fairly wide range in temperatures, its capacity for adjustment is limited. It has been stated in the literature that water drunk freely during exposure to high air temperature prevents disaster in periods of thermal stress and may even prevent a rise in body temperature, but the work³ carried on at the Pittsburgh Experiment Station of the Bureau of Mines jointly with the Research Laboratory of the American Society of Heating and Ventilating Engineers clearly demonstrates that water, even when taken in large quantities, had little or no influence in preventing a rise in body temperature in human beings.

1 Lusk, Graham, and DuBois, E F. On the Constancy of the Basal Metabolism, *J Physiol* **59** 213 (Oct) 1924.

2 Zuntz, N, and Loewry, A. *Berl klin Wchnschr* **54** 825, 1916, *Biochem Ztschr* **90** 244, 1918.

3 McConnell, W J, and Houghten, F C. Some Physiological Reactions to High Temperatures and Humidities, *J Am Soc Heat & Vent Eng* **29**, No 2, March, 1923. McConnell, W J, Houghten, F C, and Yagloglou, C P. Air Motion, High Temperatures and Various Humidities. Reactions on Human Beings, *J Am Soc Heat & Vent Eng* **30**, No 3, March, 1924.

While there is considerable material available for the study of changes in body temperature, pulse frequency and blood pressure, little work has been done on human beings showing the relation between the metabolic rate and external temperature conditions.

The difficulty in establishing this relation probably has been due to the fact that in high temperatures the wet bulb temperature of the air becomes more important than the dry bulb temperature in influencing the human body. In addition, air movement, if any, constitutes an equally important factor and in many instances the most powerful of the three. The difficulty in evaluating the relative importance to be attached to these three measurements has only been recently obviated through the development of the effective temperature index, which combines the three physical air factors into a single index.

On this index depends the comfort or discomfort of human beings, as far as atmospheric heat is concerned, and because of the fact that it also determines the physiologic effects of heat on the human body it has been called "effective temperature index."

EFFECTIVE TEMPERATURE

To form a clear conception of what effective temperature is, it will be desirable to take up briefly the development of this index. In Figure 1 the horizontal scale represents the dry bulb and the vertical scale the wet bulb temperature of the air. When the air is saturated with moisture the dry and the wet bulb temperatures have the same numerical value. Therefore, the diagonal line *ab* represents the saturation line. Suppose now that there are two chambers in which the temperature and humidity can be controlled independently of each other, and that a saturated condition of 70 degrees is produced and maintained in one of the chambers. This condition is represented in Figure 1 by *c*. If the wet bulb temperature in the second chamber is kept lower than that in the first chamber, and the feelings of warmth of the two are compared by human subjects, it will be found that its dry bulb temperature must be increased for the warmth and comfort of the two conditions to be the same. This also follows from a consideration of the fact that the human body loses heat first by radiation and convection, which depends on the dry bulb temperature of the air and of the surrounding walls and objects, and second by evaporation of moisture from its surface, the latter depending on the wet bulb depression (arithmetical difference between the dry and wet bulb temperature of the air). Thus an air condition with a dry bulb temperature of 72.5 degrees and a wet bulb temperature of 68 degrees, represented by *d*, will be found equally warm and equally comfortable as the saturated condition of 70 degrees when the comparison is made by individuals stripped to the waist and in still air.

If the wet bulb temperature in the second room is further reduced, keeping the saturated condition in the first room constant, a still higher dry bulb temperature will be required for the same degree of comfort. Thus, conditions represented by *c* and *f* will be found to be equally warm as the original condition at *c*. A line drawn through these points gives all other equivalent conditions of the same degree of warmth and comfort.

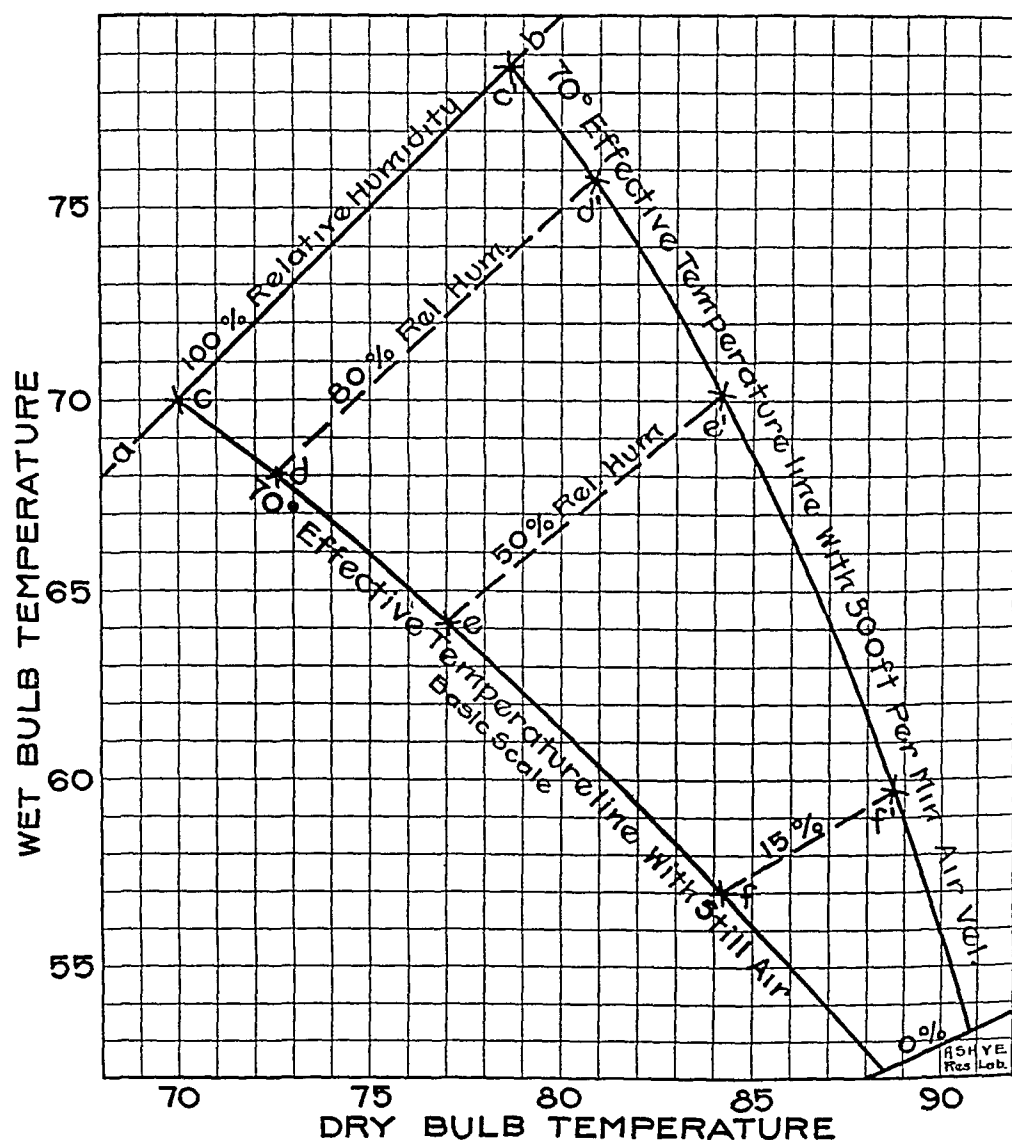


Fig 1—Equivalent conditions of temperature, humidity and air movement of 300 feet per minute, corresponding to an effective temperature of 70 degrees F

In similar manner other lines can be determined with lower and higher saturated temperature conditions in the first room, giving thus a series of lines, one for each degree of saturation temperature. The numerical value of these lines is fixed by the saturation temperature of their equivalent condition, for instance, line *cf* is the 70 degrees effective

temperature line with still air, while the next higher is the 71 degrees, and the next lower is the 69 degrees effective temperature line

In the foregoing procedure all conditions of the same effective temperature index were called "equivalent conditions of temperature and humidity" only, so far as they were determined with still air in both chambers. If now in one of the chambers the conditions represented by c , d , e and f are produced successively with still air, and a certain uniform velocity is set up in the second chamber, it will be found that with the same relative humidity in both chambers the dry bulb temperature in the velocity room must be increased to the values represented by c' , d' , e' and f' , to counterbalance the cooling effect of wind on the human body. Line $c'f'$ is the 70 degrees effective temperature line with a velocity of 300 feet per minute. In other words, all conditions on line $c'f'$ with a velocity of 300 feet per minute are equally comfortable among themselves and also equally comfortable to all conditions on line cf with still air.

The complete results of the investigation on effective temperature covering a range of from 28 to 170 degrees F dry bulb and from 25 to 115 degrees F wet bulb temperature, for human beings at rest and both stripped to the waist and normally clothed, have appeared in several issues of the Journal of the American Society of Heating and Ventilating Engineers⁴ and in other domestic and foreign publications.

With the development of the effective temperature index the effect of heat on body metabolism can be easily studied, since the factors entering into the problem can be reduced to two variables, one of which (rate of metabolism) is the independent variable.

CORRELATION OF METABOLIC RATE AND EFFECTIVE TEMPERATURE

A previous analysis⁵ of the results of several investigators, including those obtained in the psychrometric chambers of this society's Research Laboratory, disclosed that such correlation between the metabolic rate and effective temperature does exist. However, the correlation for the higher temperatures is based on the results obtained

4 Houghten, F. C., and Yagloglou, C. P. Determining Lines of Equal Comfort, *J. Am. Soc. Heat & Vent. Eng.* **29** 165-176 (March) 1923, Determination of the Comfort Zone and Further Verification of Effective Temperature Within this Zone, *ibid.* **29** 515-536, 1923, Cooling Effect on Human Beings Produced by Various Air Velocities, *ibid.* **30** 169-184 (Feb.) 1924. Yagloglou, C. P., and Miller, W. E. Effective Temperature Applied to Industrial Ventilation Problems, *ibid.* **30** 515-539 (July) 1924, Equivalent Conditions of Temperature Humidity and Air Movement Determined by Subjects Normally Clothed. Effective Temperature with Clothing, *ibid.* **31** 59-70 (Jan.) 1925.

5 Yagloglou, C. P. The Heat Given Up by the Human Body and Its Effect on Heating and Ventilating Problems, *J. Am. Soc. Heat & Vent. Eng.* **30**, No. 4, August, 1924.

on a single individual, and therefore it does not represent an average of what is expected at high temperatures

In subsequent exhaustive experiments conducted in the psychometric chambers of the Research Laboratory, seven subjects took part in the tests. To disentangle the influence of routine factors, such as food and muscular activity, on the rate of metabolism, the subjects refrained from eating for a period of eight hours prior to every experiment and abstained from any undue physical exertion.

During the first two hours of the experiment each subject rested quietly on a cot provided for the purpose in a primary room. A sample of expired air was then taken from each subject at the expiration of the two hours, and the subject was carried on the stretcher into the

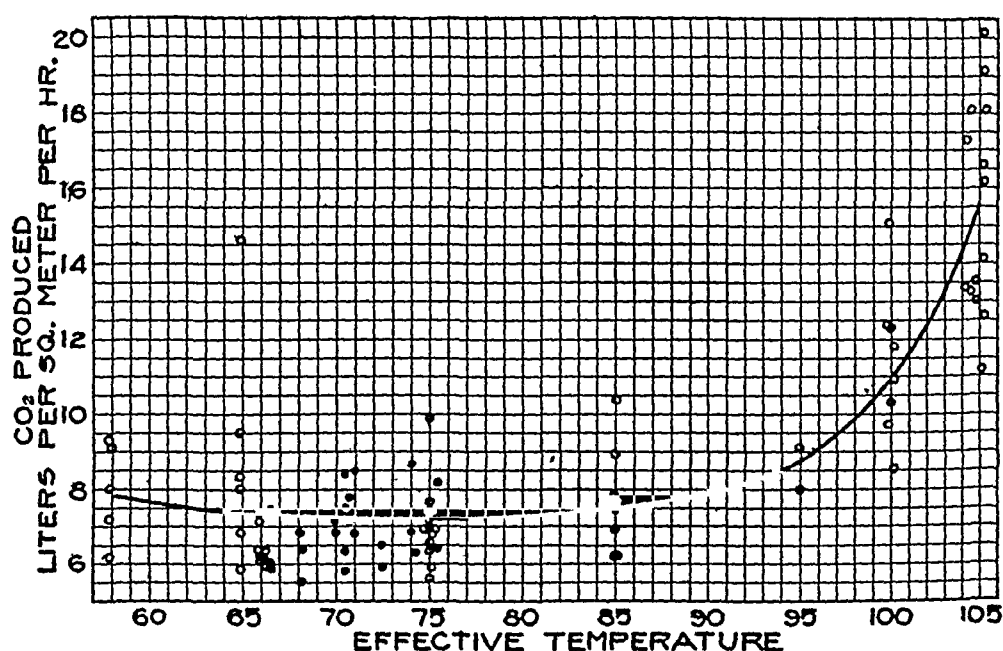


Fig 2—Relation between effective temperature and liters of carbon dioxide expired by each subject per square meter of body surface per hour

conditioned test chamber where he continued to rest in the same position. He remained in the chamber over a period varying with his ability to endure the condition. During the exposure in the chamber samples of expired air were again taken. All these experiments were conducted in still air, which was renewed at an adequate rate.

The average normal measurements for the subjects employed in the experiments are shown in Table 1. The body surface was determined from these by means of the DuBois standard chart. The subjects wore light weight union underwear, work pants and shirts, socks and shoes.

The samples of expired air were collected by means of a graduated gasometer constructed by the Bureau of Mines and placed in a room

adjoining the test chamber. Rubber tubing ($1\frac{1}{4}$ inch) connected the gasometer with the mouthpiece, the valves⁶ of which were likewise constructed by the Bureau of Mines. To become accustomed to the apparatus, the subjects breathed through the gasometer for several minutes before samples were taken. This permitted the stagnant air in the system to be forced out. Each sample collected consisted of approximately 60 liters of expired air. All volumes were reduced to 0 C temperature and dry and 760 mm of mercury barometric pressure. The ventilation rate or volume of air expired per minute was obtained by dividing the total volume by the time in minutes. From each sample collected an average sample was analyzed on a large Haldane apparatus⁷ for carbon dioxide and oxygen from which the heat developed within the body under the various temperature conditions was computed, using Zung's table of calorific equivalents of 1 liter of oxygen.

The data and results of the present series of experiments are presented in Table 2. Temperature conditions of the primary room are given at the left of Column 3, and those of the test chamber at the right

TABLE 1—*Statistics of Subjects of Experiments*

Subjects	Weight, Kg	Height, Cm	Body Surface, Sq Meters
1	61.6	163.0	1.66
2	64.5	183.1	1.84
3	69.5	191.0	1.97
4	72.0	171.3	1.84
5	59.2	167.0	1.68
6	59.4	172.8	1.72
7	71.1	174.7	1.82

of the same column. The length of exposure before taking samples is shown in Column 4. The carbon dioxide produced and the oxygen absorbed in liters per hour at 0 C temperature and 760 mm of mercury barometric pressure are shown in Columns 5 and 6, respectively.

The computed ratio of the former to the latter, or respiratory quotient, is given in Column 7. The total number of calories developed per hour is given in Column 8, from which is calculated the heat produced within the body per square meter of body surface per hour, given in Column 9. Columns 10 and 11 give the average physiologic reactions recorded during the period during which the samples were taken.

It will be observed that both the carbon dioxide output and the oxygen consumed increase with exposure to either high or low temperatures. The range of temperature employed in the experiments

6 Fulton, W. B. An Improved Air Valve for Apparatus Used in Basal Metabolism Work, *Arch Int Med* **33** 497-499 (April) 1924.

7 Burrell, G. A., and Seibert, F. M. The Sampling and Examination of Mine Gases and Natural Gas, Bull. 42, Bureau of Mines, revised by G. W. Jones, 1924, to be published.

TABLE 2—Data and Results of Experiments

Test No and Date	Sub jects	Test Room Conditions				Exposure Before Taking Sample				Carbon Dioxide Produced Liters per Hour	Oxygen Consumed Liters per Hour	Respiratory Quotient	Total Calories per Hour	Calories per Sq M Surface per Hour	Rectal Temperature, F	Pulse Rate, Beats per Minute
		Primary		Secondary		Primary Room		Secondary Room								
		Dry Bulb	Wet Bulb	Effective Temp	Dry Bulb	Wet Bulb	Effective Temp	Hours	Minutes							
37 A S 6/4/23	1	75.0	66.8	70.5	105.6	104.2	104.3	2	00			0.60	53.5	32.6	98.0	72
	1				105.6							22.14	113.1	69.2	102.1	153
	2	75.0	66.8	70.5	105.6	101.2	101.3	2	20	2	00	0.984	81.6	44.3	98.4	64
	2							0	56			1.009	138.9	87.7	102.5	158
38 A S 6/8/23	1	76.4	64.2	70.0	100.0	100.0	100.0	2	00			11.76	65.6	39.5	98.0	63
	1				100.0			1	37			16.86	81.1	49.4	101.7	132
	2	76.4	64.2	70.0	99.0	99.9	99.9	2	25	1	37	1.063	66.7	36.8	98.2	60
	2				99.0			1	14			0.929	113.7	62.7	101.8	150
39 A S 6/11/23	1	77.0	69.0	72.5	95.0	95.0	95.0	2	00			9.66	54.3	33.2	98.0	80
	1				95.0			1	54			13.20	68.6	41.9	99.3	96
	2	77.0	69.0	72.5	95.0	95.0	95.0	2	20	1	54	0.961	64.6	35.6	99.0	76
	2				95.0			2	1			0.908	92.6	51.2	100.1	96
40 A S 6/27/23	1	76.0	67.0	71.0	104.6	104.6	104.6	1	30			11.22	61.9	37.4	98.4	72
	1				104.6			1	2			29.82	137.5	83.8	102.0	140
	2	76.0	67.0	71.0	104.6	104.6	104.6	1	50	0	2	0.960	81.5	43.3	98.0	70
	2				104.6			0	54			0.963	127.8	69.5	101.8	145
41 A S 6/29/23	1	79.5	63.0	70.6	103.3	105.3	105.3	1	30			10.32	52.9	32.1	98.0	74
	1				105.3			1	00			23.16	113.5	68.7	100.8	114
	1	79.5	63.0	70.6	105.3	105.3	105.3	1	20	1	00	1.087	129.3	79.3	101.9	136
	2				105.3			1	52			0.962	71.4	39.4	99.0	70
42 A S 7/2/23	2				105.3	105.3	105.3	0	54			23.22	114.4	63.2	100.6	123
	1	77.2	65.3	70.7	105.1	105.1	105.1	1	30			11.94	61.9	37.7	97.8	80
	1				105.1			0	58			12.36	86.8	52.0	100.8	120
	1	77.2	65.3	70.7	105.1	105.1	105.1	1	18	1	58	1.200	147.1	88.6	102.3	136
43 A S 7/6/23	2				105.1	105.1	105.1	0	53			15.90	78.1	43.3	98.0	82
	2				105.1			1				26.88	139.3	75.6	101.6	141
	3	79.7	72.8	75.5	140.0	93.2	100.1	1	30			11.04	69.0	35.2	98.0	64
	3				140.0	93.2	100.1	1	21	1	58	1.041	81.6	41.7	101.1	105
44 A S 7/10/23	3	79.7	72.8	75.5	140.0	93.2	100.1	2	10	2	10	1.112	99.4	50.7	102.3	112
	2				140.0	93.2	100.1	1	50			0.951	79.3	43.2	98.0	72
	2				140.0	93.2	100.1	1	21	1	58	1.013	108.6	59.1	101.1	114
	4	80.7	69.8	74.3	104.8	104.8	104.8	2	00			0.942	61.2	33.3	99.0	70
46 A S 7/13/23	4				104.8			1	15			1.246	101.6	55.2	101.3	172
	4				104.8			1				1.381	98.7	53.7	102.2	186
	2	81.8	70.8	75.1	105.3	105.3	105.3	2	00			0.992	68.9	38.1	98.0	66
	2				105.3			0	50			0.992	140.3	76.2	101.7	134
								1	10			1.612	126.3	69.8	102.4	140

49 A S 7/20/23	1	1	82.1	68.2	71.0	100.0	99.9	99.9	0	56	11.22	12.12	0.925	60.1	26.5	98.2	75
	1	1	82.1	68.2	71.0	100.0	99.9	99.9	0	55	15.02	15.30	1.047	78.0	17.6	100.3	104
	2	2				100.0	99.9	99.9	0	40	16.90	17.28	0.920	85.5	47.3	98.4	60
	2	2				100.0	99.9	99.9	1	35	22.71	21.21	1.071	108.0	59.7	99.7	110
											27.66	28.80	0.960	143.9	79.5	102.1	100
50 A S 7/23/23	1	1				65.3	64.2	64.9	1	10	9.60	10.32	0.930	51.2	31.3	97.9	58
	1	1				65.3	64.2	64.9	3	16	13.14	13.44	0.977	67.4	41.2	96.8	56
	5	5				65.3	64.2	64.9	0	47	13.92	11.82	1.178	61.1	36.6	99.2	63
	5	5				65.3	64.2	64.9	3	46	24.48	15.81	1.519	90.3	53.4	97.6	62
	6	6				65.3	64.2	64.9	0	18	11.52	12.00	0.960	60.0	35.3	98.0	70
	6	6				65.3	64.2	64.9	3	14	16.08	13.98	1.150	73.1	43.3	97.3	68
51 A S 7/24/23	1	1				75.2	74.7	75.0	1	31	9.66	9.30	1.039	47.4	29.2	98.2	64
	1	1				75.2	74.7	75.0	4	36	10.38	10.08	1.029	51.2	31.2	98.0	60
	3	3				75.2	74.7	75.0	1	65	10.98	11.16	0.985	56.1	28.7	97.9	64
	3	3				75.2	74.7	75.0	5	00	13.50	11.70	1.152	61.8	31.3	97.6	64
	6	6				75.2	74.7	75.0	2	4	11.70	11.76	0.995	59.3	35.0	98.2	63
	6	6				75.2	74.7	75.0	5	11	11.76	12.12	0.970	60.7	35.8	97.8	68
52 A S 7/25/23	6	6				84.7	83.3	85.0	2	14	11.22	10.62	1.057	54.4	32.0	98.1	70
	6	6				84.7	83.3	85.0	3	59	16.86	11.82	1.427	65.7	38.9	97.8	64
	6	6				84.7	83.3	85.0	4	57	13.08	11.34	1.183	59.4	35.1	97.8	64
	3	3				84.7	83.3	85.0	1	58	13.38	12.00	1.115	62.2	31.7	98.0	62
	3	3				84.7	83.3	85.0	3	45	14.46	14.04	1.030	71.4	36.5	98.4	64
	3	3				84.7	83.3	85.0	4	43	13.98	12.30	1.138	64.1	32.9	98.4	66
53 A S 7/27/23	3	3				102.3	77.8	85.0	2	32	12.24	12.06	1.015	61.0	31.1	98.6	70
	3	3				102.3	77.8	85.0	5	8	12.24	11.70	1.048	69.7	30.5	98.6	70
	6	6				102.3	77.8	85.0	2	2	12.72	11.28	1.129	58.6	24.5	98.7	68
	6	6				102.3	77.8	85.0	4	52	15.12	13.38	1.130	68.0	39.7	98.5	70
54 A S 7/28/23	3	3				85.0	85.0	85.0	1	23	13.56	11.88	1.141	62.0	31.7	98.1	66
	3	3				85.0	85.0	85.0	4	11	15.06	12.24	1.230	65.2	33.3	97.8	64
	6	6				85.0	85.0	85.0	1	23	17.76	12.60	1.110	69.8	40.8	98.5	72
	6	6				85.0	85.0	85.0	4	6	13.20	10.62	1.243	56.7	33.6	98.2	66
1 L 7/10/24	1	1							1	30	10.56	11.76	0.897	57.9	35.3	98.2	72
	1	1				67.6	64.3	66.0	0	59	10.02	11.22	0.893	55.2	33.6	97.7	66
	1	1				67.6	64.3	66.0	2	21	10.50	11.61	0.902	57.4	35.0	97.5	60
	7	7				67.6	64.3	66.0	1	40	10.14	12.12	0.837	58.8	32.0	98.9	62
	7	7				67.6	64.3	66.0	1	26	11.28	13.26	0.851	64.5	35.1	98.2	58
	7	7				67.6	64.3	66.0	2	48	11.28	13.86	0.848	67.3	36.6	98.0	57
	6	6				67.6	64.3	66.0	1	50	11.58	13.44	0.862	65.6	38.7	98.4	73
	6	6				67.6	64.3	66.0	1	49	12.24	13.86	0.883	68.0	40.3	98.4	70
	6	6				67.6	64.3	66.0	3	7	12.54	14.46	0.897	70.7	41.8	98.2	69
2 L 7/11/24	1	1							1	10	9.72	10.74	0.905	53.0	32.3	98.0	72
	1	1				79.4	53.7	57.9	1	3	11.88	13.50	0.850	66.2	40.3	98.0	56
	7	7				59.4	55.7	57.9	1	40	10.98	12.06	0.897	61.8	33.6	98.4	59
	7	7				59.4	55.7	57.9	1	31	11.34	12.12	0.935	60.2	32.7	98.3	52
	7	7				59.4	55.7	57.9	2	57	17.28	17.10	1.012	89.7	47.2	98.4	54
	6	6				59.4	55.7	57.9	2	10	12.72	13.32	0.855	66.5	38.8	98.4	76
	6	6				59.4	55.7	57.9	2	52	13.50	14.58	0.926	72.3	42.7	97.8	68
	6	6				59.4	55.7	57.9	2	2	15.18	17.01	0.908	84.1	49.6	97.7	68

varied from about 55 to 130 degrees effective temperature, but the table includes results only up to about 105 degrees. For temperatures above 105 degrees effective temperature, the subjects could not endure the condition long enough to obtain a representative sample, which required at least an hour's exposure, and therefore the few results obtained above 105 degrees are not included herein.

CARBON DIOXID PRODUCED AND OXYGEN CONSUMED

To afford a uniform basis of comparison between different subjects, the carbon dioxide produced and the oxygen consumed are expressed in liters per square meter of body surface per hour. The variations of these quantities with effective temperature are shown in Figures 2 and 3, respectively.

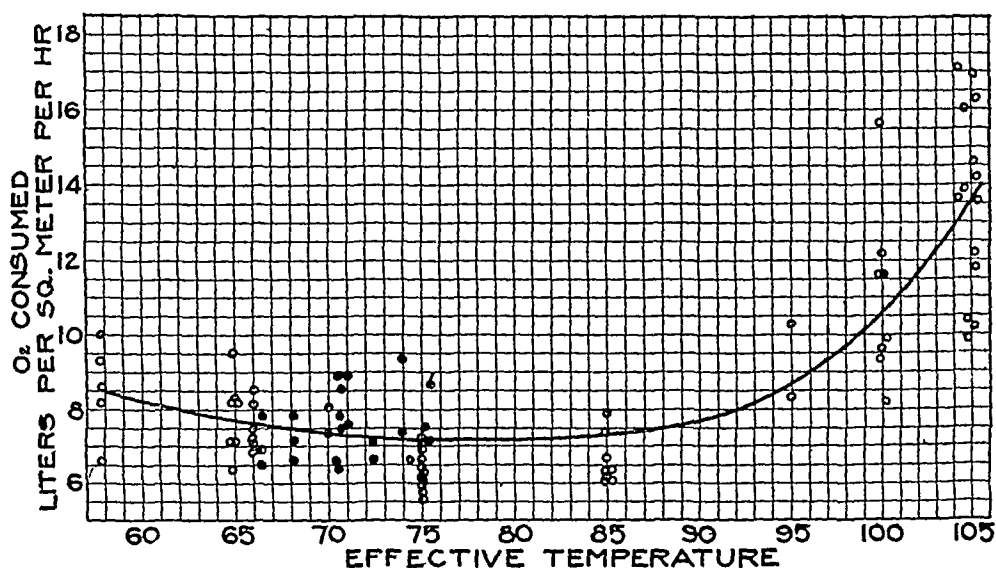


Fig 3—Relation between effective temperature and liters of oxygen consumed by each subject per square meter of body surface per hour

Black circles represent observations made in the primary room, and white or open circles those made in the test chamber. These graphs are very similar and have the same characteristics. A minimum value of 7.2 liters per square meter of body surface per hour is obtained both within a temperature zone of between 70 and 85 degrees effective temperature, where the rate of gaseous exchange is measurably constant. Above and below this zone both quantities increase at an accelerated rate. At the ordinary temperature of 65 degrees effective temperature, the figures show an average of 7.3 liters of carbon dioxide expired and 7.7 liters of oxygen consumed per square meter of body surface per hour. This corresponds to a respiratory quotient of 0.948.

Above 85 degrees effective temperature, the rate of gaseous exchange increases rapidly, and with a still greater rate when the body temperature is exceeded.

It will be observed that in these experiments the respiratory quotient varied from about 0.84 to 1.55. The relation of this ratio to effective temperature, as computed from the average values given in Figures 2 and 3, is shown in Figure 4.

As the effective temperature increases, the respiratory quotient increases also until at about 80 degrees effective temperature it becomes unity. Between 80 degrees and body temperature the variation in the respiratory quotient is rather small, but above body temperature a very sudden increase is apparent, according to the limited results available at these high temperatures. Because of the insufficient number of tests this portion of the curve is shown dotted.

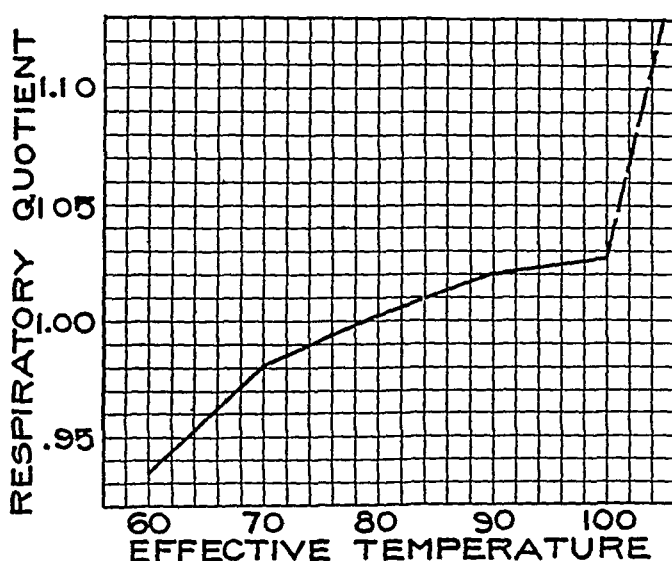
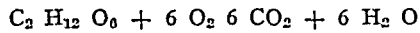


Fig. 4—Relation between respiratory quotient and effective temperature

EFFECT OF HIGH TEMPERATURES ON THE BODY AND RESPIRATORY QUOTIENT

Within the ordinary range of temperature conditions the respiratory quotient, as recorded by various investigators, seldom exceeds unity. The question now arises as to how high temperatures affect the human body so as to raise the respiratory quotient over unity. Theoretical considerations suggest that free oxygen is available in the body through its liberation during the transformation of carbohydrates into fats. A study of the respiratory exchanges of animals which are rapidly laying on a store of fat at the expense of a carbohydrate diet indicates that oxygen is set free. Thus, the marmot eats large quantities of carbohydrate food toward the end of the summer, and rapidly lays on a thick layer of subcutaneous fat to last it during the winter.

Starling⁸ points out that a considerable loss of oxygen is incurred in the formation of fat from carbohydrate. He states that if glucose were entirely oxidized in the body the amount of oxygen absorbed would be exactly equal to the amount of carbon dioxide involved. Thus,



In this case the respiratory quotient would be $\frac{6 CO_2}{6 O_2} = 1$

If, however, oxygen is being set free by the conversion of part of the carbohydrate into fat, this oxygen will be available for the oxida-

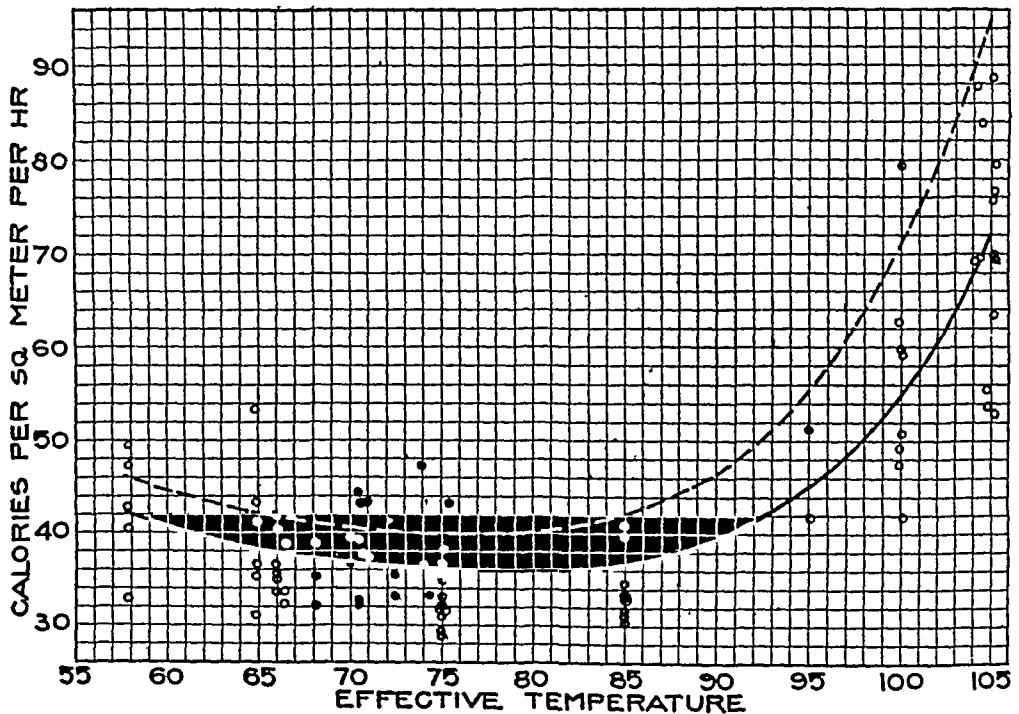


Fig 5—Calories produced per square meter of body surface per hour at various effective temperatures

tion of other portions of the carbohydrates. The animal will not require so much oxygen from external sources for the production of the same amount of carbon dioxide, and therefore the carbon dioxide output of the animal will be greater than its oxygen intake. Pembrey⁹ has shown that under this condition the respiratory quotient may be as high as 1.50.

As an alternative hypothesis, it is just as possible that the high respiratory quotient is due to the forced loss of excessive carbon dioxide from the body during exposure. This loss may be either through sweat or through increased breathing, or both.

⁸ Starling, E. H. Principles of Human Physiology, Ed 3, 1920, pp 826-938

⁹ Pembrey, J. Physiol 27 407, 1901, cited by Starling (Footnote 8)

The heat produced within the body in calories per square meter of body surface per hour calculated in the manner shown previously is given in Figure 5, plotted against effective temperature. At 65 degrees effective temperature the average subject of the experiments developed 38.2 calories per square meter of body surface per hour. This value checks closely the DuBois' standard for basal metabolism—38.6 calories—but the curve shows that it is by no means the minimum metabolism.

The temperature zone of minimum heat production appears to be between 75 and 83 degrees effective temperature. It will be observed that within this range the lowest value of 36 calories per square meter per hour is reached. This belief is substantiated from results of various

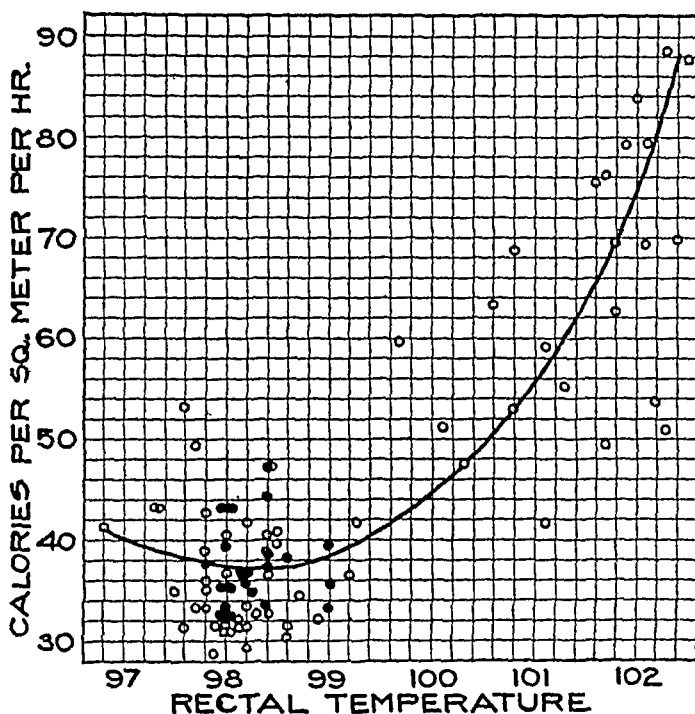


Fig 6—Correlation between heat production and rectal temperature

other investigators who recorded values well below DuBois' standard, depending undoubtedly on the temperature in which the observations were made.

THERMOSTATIC CONTROL OF THE BODY

Attention is called to the large temperature range of successful operation of the body thermostatic control, within which the heat production and heat loss are accurately balanced. The most economical range is represented by the flat portion of the curve for ordinary atmospheric conditions. Above 85 degrees effective temperature, however, there is apparently a stress on the mechanism. The body makes strenuous efforts to resist rise in its temperature by promoting evaporation of perspiration from its surface, but the limit of adjustment fails

above 90 degrees effective temperature. This is indicated by the rapid increase both of heat production and of body temperature at the higher temperatures. At 105 degrees effective temperature the heat production is twice as great as at the ordinary temperature of 65 degrees.

A tendency for an increase in heat production is also shown below 65 degrees effective temperature, which heat is necessary to keep the body warm in cold surroundings.

INFLUENCE OF FOOD AND POSTURE ON BODY METABOLISM

To study the influence of food and sitting position on body metabolism it is proposed to introduce here the previous study on metabolism mentioned above.⁵ In Figure 5 the dotted line represents the relation

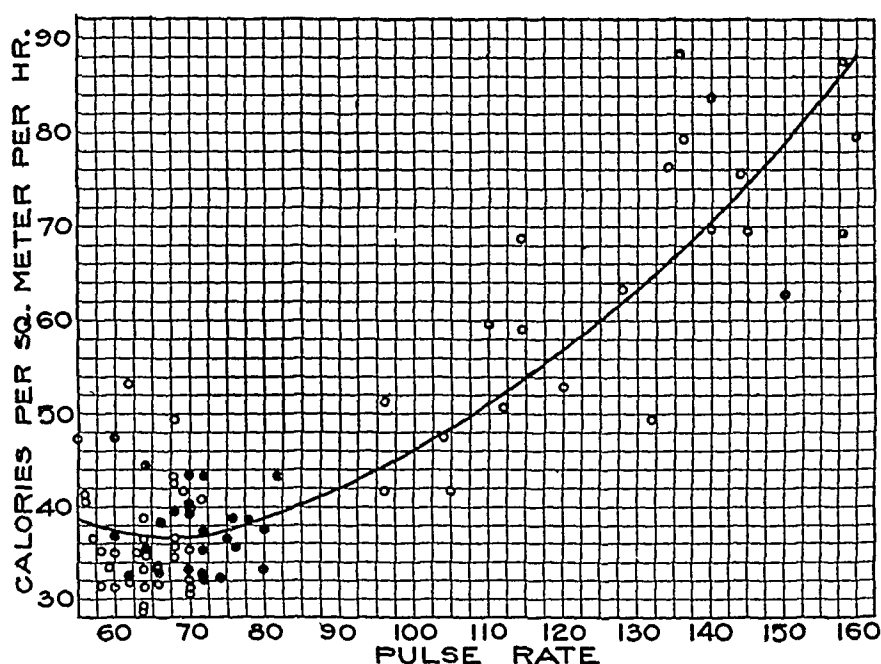


Fig 7—Correlation between heat production and pulse rate

between the metabolic rate and the effective temperature of the exposure when regular meals were partaken and the subjects sat comfortably on chairs. The experimental data from which this relation was drawn are largely based on the results obtained by indirect calorimetry on a subject well trained to the routine of experiments. The respiratory samples were analyzed for carbon dioxide and oxygen on a large Haldane gas apparatus, and the heat production was calculated therefrom after reducing all volumes to 0 C and dry and 760 mm of mercury barometric pressure. Since the experiments under consideration were conducted practically under the same condition of clothing and exposure as those presented in this paper, the difference between the two curves in Figure 5 represents the increase in metabolism due to food and sitting

position The two curves are practically parallel within the ordinary range of temperature and their difference amounts to about 4 calories per square meter per hour In other words, the metabolism at the ordinary temperature of 65 degrees increases by 11 per cent over the basal value when the subjects partake of their regular diet and sit comfortably on chairs

For the higher temperatures exceeding 80 degrees effective temperature, the increase in metabolism through food and sitting position is not constant but is accelerated as shown by the divergence of the two curves

It is of interest to note that the temperature zone of minimum metabolism, or the most economical thermal zone of the human body, is included very nearly between the same two temperature limits for both curves These two limits may possibly vary with the amount of clothing worn, but no great variation is expected, owing to the fact that it was found in another investigation that clothing makes little or no difference in the thermal condition of the human body when the temperature of the environment exceeds 90 degrees effective temperature

CORRELATION OF RECTAL TEMPERATURE AND PULSE RATE TO METABOLIC RATE

An examination of Table 2 will show that whenever more than one sample of expired air was taken in the test chamber at different periods of exposure the heat production invariably increased with the time of exposure This is to be expected when it is considered that the physiologic reactions vary with temperature and time of exposure Accordingly, an attempt was made to correlate the rate of metabolism with rectal temperature and with pulse rate in Figures 6 and 7, respectively

It will be observed that the rate of metabolism correlates fairly well with these two physiologic reactions In Figure 6 the heat production becomes minimal at a body temperature of about 98.4 degrees F and it increases both above and below this temperature It is obvious that metabolism must necessarily increase when there is a drop in body temperature, to keep the body warm The increase at the higher temperatures is attributed to the warming up of the tissues, which speeds up their chemical activities, and the marked rapid increase for temperatures above 100 degrees is apparently allowed by the breaking down of the human thermostatic control

Similarly it will be noted that the heat production attains a minimum value of about the same magnitude as shown in the previous figure, namely, 37 calories per square meter per hour, at a pulse rate of 68 beats per minute Metabolism again increases with higher or lower

pulse rates, but the rate of increase is not as great as it is with change in temperature. A comparison of Figures 6 and 7 shows that the pulse curve is much flatter than that for temperature, indicating that the former is a more dependable direct index of the metabolic rate. The pulse rate increases approximately in direct proportion to the metabolic rate, therefore, the heart works just fast enough to supply the tissues with the extra oxygen needed.

SUMMARY AND CONCLUSIONS

Dry and wet bulb temperatures (and velocity of air, if any) have been shown to be reducible to one single index called effective temperature. (The charts and tables for determining the effective temperature from the three factors will simplify the study of similar problems.)

The results of these experiments lead to the following conclusions:

- 1 The rates at which oxygen is consumed and carbon dioxide is produced increase with exposure to either high or low temperatures.

- 2 There is a zone of minimum metabolic rate between 75 and 83 degrees effective temperature within which basal metabolism should be measured.

- 3 The metabolic rate increases rapidly when the temperature of the environment is higher than that of the body.

- 4 Body temperature and pulse rate correlate fairly well with the rate of basal metabolism.

THE METABOLISM OF OBESITY

IV THE DISTRIBUTION OF ENERGY PRODUCTION AFTER FOOD *

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Previous work has shown that there is no demonstrable dependence of obesity upon food intake or caloric balance ¹ Neither can it be proved that any consistent relation exists between constitutional obesity and basal metabolism ² It has been shown, however, that these people show a lower specific dynamic action of protein following a high protein intake than normal and thin people ³ The work here reported was done in conjunction with our investigations on basal metabolism and specific dynamic action of foods While the figures of this work, based, as they are on the urinary nitrogen and the nonprotein respiratory quotient may not be considered as absolutely exact, attention is called to the fact that their relative value is unimpaired because conditions were so well controlled Each subject served for at least three tests, which were conducted by the same person and under the same conditions except for the type of meal served

EXPERIMENTS

Tests were made on obese, thin and normal people, using the same protein, carbohydrate and fat meals described in our previous article ³ Half an hour before the basal metabolism was taken, the subject was asked to empty the bladder completely Immediately before the meal, the first urine specimen was collected, and again following each test The nitrogen content of each specimen was determined by the micro-Kjeldahl method of Folin and Denis ⁴ From this the nonprotein

* From the Medical Clinic, the Gusta Morris Rothschild Fund and the Otto Baer Fund for Clinical Research of the Michael Reese Hospital and the Nelson Morris Memorial Institute for Medical Research

1 Strouse, S, and Dye, M Studies on the Metabolism of Obesity I The Relation Between Food Intake and Body Weight in Some Obese Persons, Arch Int Med **34** 267 (Sept) 1924

2 Strouse, S, Wang, C C, and Dye, M Studies on the Metabolism of Obesity II Basal Metabolism, Arch Int Med **34** 275 (Sept) 1924

3 Wang, C C, Strouse, S, and Saunders, A D Studies on the Metabolism of Obesity III The Specific Dynamic Action of Food, Arch Int Med **34** 573 (Oct) 1924

4 Folin, O, and Denis W Nitrogen Determinations by Direct Nesslerization I Total Nitrogen in Urine, J Biol Chem **26** 473 (Sept) 1916

respiratory quotient for each particular period was determined according to Lusk,⁵ and the proportion of carbohydrate and fat used as energy during that period was computed from the table of Zuntz and Schumberg, as modified by Lusk. The calories derived from nitrogen were computed also from Lusk's figures, each gram of urinary nitrogen representing 26.51 calories.

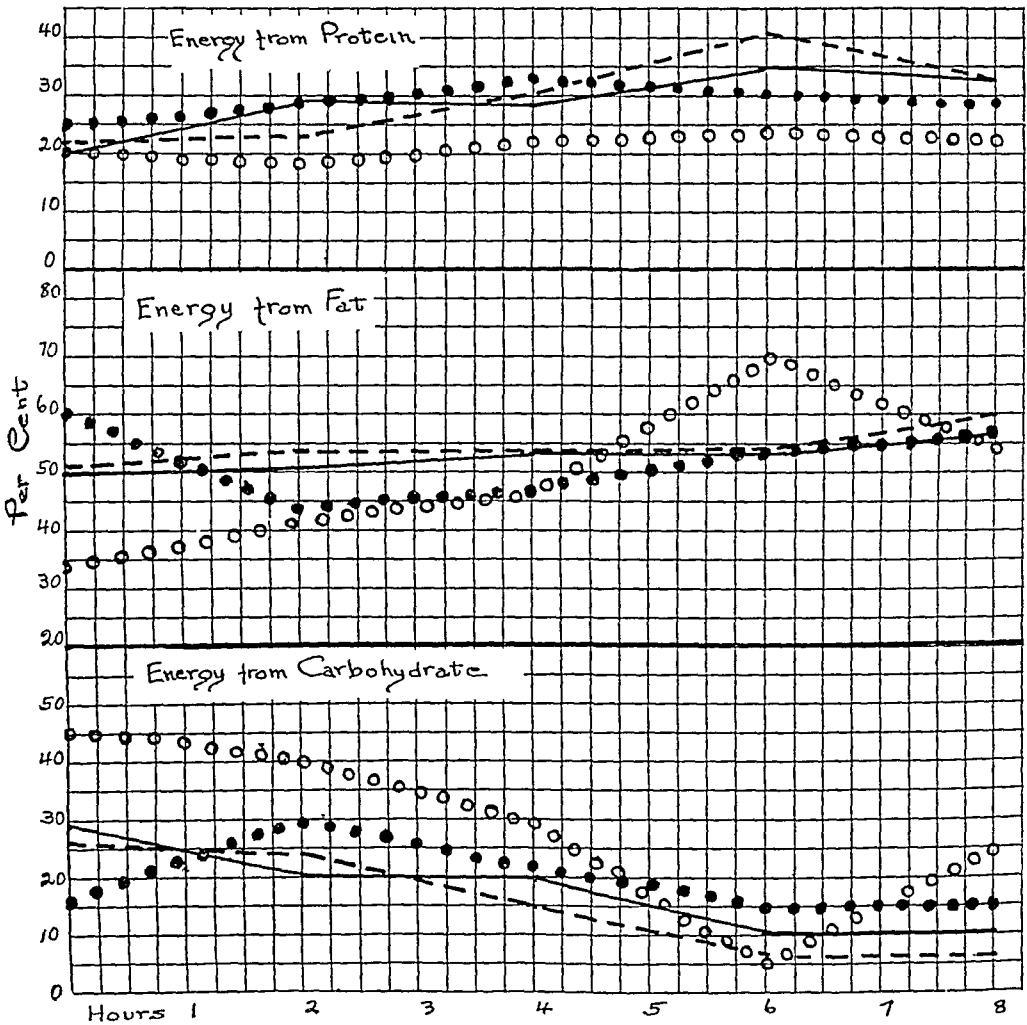


Fig 1

Chart 1—Average percentage distribution of energy production after a high protein meal. Solid dot, overweight, Group 1, open dot, overweight, Group 2, solid line, underweight, and broken line, normal.

Nine obese subjects varying in weight from 37 to 97 per cent above normal served in eleven tests with the protein meal. With the exception of a 13 year old girl and a 16 year old boy, the subjects were all young women. Their actual weight varied between 76.4 and 118.1 kg.

⁵ Lusk, G. The Science of Nutrition, Ed 3, Philadelphia, W. B. Saunders Company, 1921.

COMMENT

In comparing results of the work with obese people, it will be noted that these can be divided into two distinct groups (Table 1). The first of these, comprising seven people and eight tests, or 78 per cent of the cases, after the ingestion of a protein meal showed increased energy production from carbohydrate.

In this group the average basal heat production from the carbohydrate was 16 per cent of the total basal heat production, after two hours it became 29 per cent of the total, after four hours 22 per cent, after six hours 15 per cent, and at the end of the eight hour period it was still 15 per cent of the total calories produced. Group 2, on the other hand, in which three tests were made on two people, showed a decrease in the calories produced from the carbohydrate after the ingestion of a protein meal averaging 46 per cent of the total basal heat production, followed by 40, 28, 5 and 24 per cent in the subsequent two hour periods. One of the two subjects in this group on whom two tests were made was not perhaps really obese. She was a girl, aged 13, suffering from gigantism, and was very large for her age but quite solid.

Of the eight thin subjects, only one had increased heat production from carbohydrate or such a slight increase that it was within experimental error. The averages showed a decided decrease from the basal to the end of the experiment, namely, 29, 21, 19, 11 and 11 per cent in the five periods. The curve for the normal subjects is very much like that for the underweights. Only one of the six tests showed increased energy production over the basal during later periods. The averages were 27 per cent of the total basal and 24, 15, 6 and 7 per cent of the total heat production for the four two hour periods.

Table 2 shows an interesting relation between the calories derived from fat and those from carbohydrate. In both groups of obese there is a complementary relation, that is, where the curve for carbohydrate goes up that for fat goes down, and vice versa. The first group, which showed an increase in calories derived from carbohydrate, accompanied this by a decrease in calories derived from fat, the second group used fat in place of carbohydrate for energy. The average of calories derived from fat for the first group showed a decrease from 60 per cent in the basal to 44 per cent in the first test, and 56 per cent at the end of the period. The second group showed average values of 34, 42, 47, 70 and 54 per cent in the respective periods. The underweights and normals, however, showed very little change in the percentage of calories derived from fat from period to period. For the underweights the average figures are 50 per cent for the basal, 51, 53, 54 and 56 per cent in the subsequent periods, figures for normals are 51, 53, 54, 53 and 60 per cent.

TABLE 1—*Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Protein Meal*
Calories from Carbohydrate

Name	Sex*	Age	Height, Cm	Weight		Protein Intake, Gm	Heat Production											
				Kilo grams	Percentage of Difference from Normal		Basal		2 Hours after Meal		4 Hours after Meal		6 Hours after Meal		8 Hours after Meal			
							Calories per Hour	Per centage of Total	Calories per Hour	Per centage of Total	Calories per Hour	Per centage of Total	Calories per Hour	Per centage of Total	Calories per Hour	Per centage of Total		
M N	Q	29	155.0	93.6	+72	36.8	1	7	8	17	5	11	0	0	3	5		
S A	Q	25	149.5	82.3	+56	44.8	19	29	38	51	14	21	5	8	9	14		
L S	Q	34	165.0	118.1	+91	53.8	1	1	29	28	3	3	1	1	4	4		
A D	Q	16	169.5	84.6	+37	66.4	29	37	46	54	62	69	40	48	35	42		
R K	Q	26	148.5	76.4	+45	42.6	16	22	22	23	20	27	22	33	13	17		
K K	Q	27	148.5	75.5	+43	46.8	11	18	10	15	12	17	1	1	14	22		
P P	Q	32	171.1	103.2	+58	39.1	0	0	10	14	11	14	1	1	3	4		
L S	Q	28	165.0	85.5	+42	32.0	7	10	13	17	6	9	—	—	—	—		
Average		27	159.0	89.9	+56	45.3	11	16	22	29	17	22	12	15	12	15		
F P	Q	34	163.8	101.2	+61	60.3	31	39	9	39	18	22	3	4	47	55		
L M	Q	13	160.5	97.8	+96	41.6	42	53	31	37	16	19	7	6	5	6		
L M	Q	13	161.5	98.6	+97	44.5	—	—	38	43	37	43	3	4	8	10		
Average		20	161.9	99.2	+86	50.5	37	46	33	40	24	28	4	5	50	24		
M A	Q	25	166.2	47.3	-22	35.8	20	42	7	12	15	26	5	9	10	21		
M A	Q	25	163.2	47.3	-22	64.9	15	31	11	20	6	10	7	12	5	9		
B McC	Q	31	140.0	47.3	-28	43.9	14	25	22	32	12	17	13	21	9	14		
S S	Q	42	173.4	50.2	-31	68.2	19	33	14	19	10	14	2	3	0	0		
B B	Q	21	159.4	41.6	-26	78.3	22	42	5	9	19	32	12	19	13	22		
Z O	Q	24	159.4	45.0	-19	41.5	13	26	19	30	17	28	11	20	9	16		
J W	Q	35	172.5	61.4	-11	60.8	8	13	15	21	14	20	4	6	4	7		
H B	Q	19	167.6	51.0	-14	59.1	12	22	18	23	2	4	0	0	0	0		
Average		29	163.8	48.9	-22	55.3	15	29	14	21	12	19	7	11	6	11		
M S	Q	21	167.2	62.3	+3	53.5	12	21	23	41	13	18	1	2	3	5		
M S	Q	21	167.2	61.4	+2	61.1	11	34	21	17	15	21	1	2	6	10		
M W	Q	24	160.0	65.5	+1	53.1	21	32	29	38	26	35	2	3	10	14		
D K	Q	22	163.5	59.6	+0	63.8	21	36	23	32	6	9	18	19	4	6		
J S	Q	21	161.0	57.7	+3	68.3	4	7	7	1	1	1	0	0	2	3		
L T	Q	22	163.9	54.2	-7	67.1	18	30	7	10	5	8	6	11	3	5		
Average		22	163.8	58.3	+0.5	61.1	16	27	18	24	11	15	4	6	5	7		

* In this and the following tables, ♂ indicates male, ♀ female

TABLE 2—Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Protein Meal
Calories from Fat

Name	Sex	Age	Height, Om	Weight		Protein Intake, Gm	Heat Production											
				Kilo- grams	Percentage of Difference from Normal		Basal		2 Hours after Meal		4 Hours after Meal		6 Hours after Meal		8 Hours after Meal			
							Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total
M N	♂	29	155 0	93 6	+72	36 8	57	19	41	14	32	21	48	38	62			
S A	♂	25	149 5	82 3	+56	44 8	35	54	16	31	46	39	59	31	49			
F S	♀	34	165 0	118 1	+91	53 8	87	82	52	61	70	77	79	72	74			
A D	♂	16	169 5	84 6	+37	66 4	35	44	24	7	8	21	25	25	30			
R K	♂	26	148 5	76 4	+45	42 6	34	47	43	23	32	26	39	32	42			
R K	♂	27	148 5	75 5	+43	46 8	39	64	62	43	61	54	73	36	56			
P P	♂	32	171 1	103 2	+58	39 1	48	45	62	47	61	47	73	54	75			
E S	♀	28	165 0	85 5	+42	32 0	37	55	50	42	60	42	—	—	—			
Average		27	159 0	89 9	+56	45 3	45	60	44	34	47	41	54	41	56			
F P	♂	34	163 8	101 2	+64	65 3	33	41	52	46	51	52	67	17	20			
E M	♀	13	160 5	97 8	+96	41 6	22	28	39	47	56	55	70	57	72			
E M	♀	13	161 5	98 6	+96	44 5	—	—	34	29	33	59	74	57	70			
Average		20	161 9	99 2	+86	50 5	33	34	42	41	47	55	70	44	54			
M A	♂	25	166 2	47 3	-22	35 8	15	32	25	27	47	28	53	20	42			
M A	♂	25	166 2	47 3	-22	64 9	24	49	53	37	64	31	54	30	57			
B McC	♂	31	170 0	47 3	-28	43 9	35	64	51	43	61	33	51	39	62			
S S	♂	42	173 4	50 2	-31	58 2	27	47	69	43	58	43	65	49	73			
B D	♂	21	159 4	41 6	-26	78 3	23	43	40	27	44	27	44	29	48			
Z O	♂	24	159 4	45 0	-19	41 5	32	62	58	33	51	34	61	36	65			
J W	♂	35	172 5	61 4	-11	60 8	36	57	56	32	45	30	50	32	57			
H B	♂	19	167 6	51 0	-14	59 4	27	50	39	30	48	29	48	24	44			
Average		29	166 8	48 9	-22	55 3	27	50	51	34	53	32	54	32	56 *			
M S	♂	21	167 2	62 3	+3	53 5	35	60	36	40	56	35	51	38	58			
M S	♂	21	167 2	61 4	+2	53 1	29	47	45	33	45	32	52	31	53			
M W	♂	24	160 0	55 5	+1	63 8	32	49	51	31	41	45	67	43	61			
D K	♂	22	163 5	57 7	+0	65	28	47	60	47	65	41	46	45	68			
J S	♂	21	161 0	54 2	+3	67 1	39	65	60	60	72	41	62	45	67			
E T	♂	22	163 8	54 2	-7	67 1	22	36	60	28	46	23	40	29	51			
Average		22	163 9	58 3	+0 5	61 1	31	51	53	40	54	35	53	33	60			

TABLE 3—*Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Protein Meal*
Calories from Protein

Name	Sex	Age	Height, Cm	Weight		Protein Intake, Gm	Heat Production														
				kilo grams	Percentage of Difference from Normal		Basal			2 Hours after Meal			4 Hours after Meal			6 Hours after Meal			8 Hours after Meal		
							Calories per Hour of Total	Per centage of Total	Calories per Hour	Calories per Hour of Total	Per- centage of Total	Calories per Hour	Calories per Hour of Total	Per- centage of Total	Calories per Hour	Calories per Hour of Total	Per- centage of Total	Calories per Hour	Calories per Hour of Total	Per- centage of Total	
M N	♂	29	155 0	93 6	+72	36 8	15	26	19	41	25	57	23	52	20	33	23	52	20	33	
S A	♂	25	149 5	82 3	+56	44 8	11	17	25	33	23	33	22	33	23	37	22	33	23	37	
L S	♂	34	165 0	118 1	+91	53 8	18	17	19	19	22	27	20	20	20	21	20	20	20	21	
A D	♂	16	169 5	84 6	+37	60 4	16	20	19	22	21	23	23	27	24	28	23	27	24	28	
R K	♂	26	148 5	76 4	+45	42 6	22	31	23	29	30	41	30	41	31	41	19	28	31	41	
R K	♂	27	148 5	75 5	+43	46 8	11	18	16	24	16	23	19	26	14	22	14	26	15	22	
P P	♂	32	171 1	103 1	+58	39 1	24	33	18	25	18	24	19	26	13	21	19	26	13	21	
L S	♂	28	165 0	85 5	+42	32 0	24	35	24	33	22	31	22	—	—	—	—	—	—	—	
Average		27	159 0	89 9	+56	45 3	18	25	21	29	23	33	21	31	21	29	21	31	21	29	
F P	♀	31	163 8	101 2	+64	65 3	16	20	7	9	17	21	17	27	21	25	21	27	21	25	
E M	♀	13	160 5	97 8	+66	41 6	16	20	18	21	18	21	18	23	17	22	18	23	17	22	
L M	♀	13	161 5	98 6	+97	44 5	—	—	20	23	21	24	21	23	16	20	18	23	16	20	
Average		20	161 9	99 2	+86	50 5	16	20	15	18	19	22	19	24	18	22	19	24	18	22	
M A	♂	25	166 2	47 3	-22	35 8	12	26	37	63	15	26	15	38	20	38	20	38	18	38	
M A	♂	25	166 2	47 3	-22	64 9	10	20	15	27	15	26	15	33	19	34	19	33	18	34	
B McC	♂	31	170 0	47 3	-28	43 9	6	11	12	17	15	21	15	25	15	24	15	25	15	24	
S S	♂	42	173 4	50 2	-31	58 2	11	19	16	22	21	28	21	32	21	27	21	32	18	27	
B D	♂	21	159 4	41 6	-26	78 3	8	15	13	22	14	24	14	37	18	30	13	37	18	30	
Z O	♂	24	159 4	45 0	-19	41 5	6	12	7	12	11	19	11	20	10	18	11	20	10	18	
J W	♂	35	172 5	61 4	-11	60 8	20	31	16	23	25	35	27	44	20	36	27	44	20	36	
H B	♂	19	167 6	51 0	-14	59 4	16	29	21	34	31	49	31	52	30	66	31	52	30	66	
Average		29	166 8	48 9	-22	55 3	11	20	17	28	18	29	21	35	18	33	21	35	18	33	
M S	♂	21	167 2	62 3	+3	53 5	11	19	16	23	19	26	19	47	24	37	32	47	24	37	
M S	♂	21	167 2	61 4	+2	61 1	12	19	13	17	25	35	29	47	22	37	29	47	22	37	
M W	♂	24	160 0	65 5	+1	53 1	13	17	13	17	18	24	20	30	17	24	20	30	17	24	
D K	♂	22	163 5	58 6	+0	63 8	10	17	12	17	19	26	19	35	17	28	24	35	17	28	
E T	♂	22	163 8	54 2	-7	67 1	20	34	20	30	29	46	29	38	25	44	29	38	25	44	
J S	♂	21	161 0	57 7	+3	68 3	17	28	19	28	22	27	22	38	19	30	22	38	19	30	
Average		22	163 8	58 3	+0 5	61 1	14	22	16	23	22	31	27	41	21	33	27	41	21	33	

The actual calories derived from protein, as seen in Table 3, vary little in the two groups of obese subjects. There is likewise little difference in the percentage of calories derived from protein in these groups, varying in the first group between 25 per cent as a basal and 33 per cent, the highest point four hours later, and in the second group a basal of 20 per cent with the highest point, 24 per cent, occurring six hours later. Both the underweight subjects and the normals showed a decided rise in the percentage of calories from protein. The average basal percentage of calories from protein for the underweight subjects was

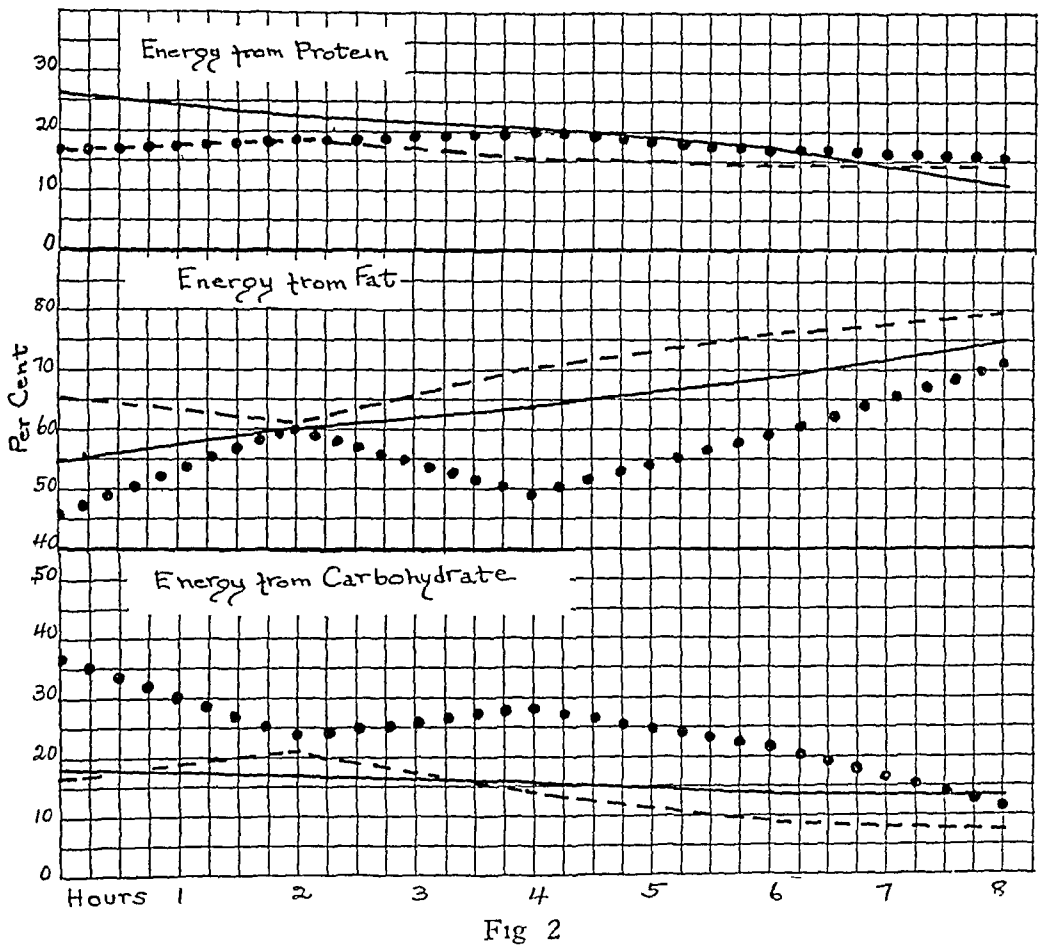


Chart 2—Average percentage distribution of energy production after a high fat meal. Solid dot—overweight, solid line—underweight, and broken line—normal.

20, followed by 28, 29, 35 and 33 per cent in the subsequent periods. The corresponding basal figures for normals were 22 per cent and in the following periods 23, 31, 41 and 33 per cent.

The fact that 82 per cent of the obese cases showed a decided rise in calories derived from carbohydrate and a decrease in calories derived from fat after a high protein meal, and that the thin and normal subjects, on the other hand, utilized protein and fat as their chief sources of

energy, is rather suggestive as to a possible reason for obesity and thinness. The obese conserve the body fat and thus accumulate more, whereas the normal and thin people use up their food fat instead of storing it. On a high protein meal, the normals and the thin people also use much of the protein for energy production but the obese do not. The basal, however, shows that the obese derive most of their energy from fat while on starvation, but the thin and normal have no such store of fat and therefore use up more carbohydrate. Possibly the latter may store more of their carbohydrate as glycogen while the obese convert theirs into fat at once.

Tables 4, 5 and 6 represent what happens after the ingestion of a carbohydrate meal. All subjects used more carbohydrate after the ingestion of 100 gm of sucrose. The calories derived from carbohydrate during the basal period in obese people was 20 per cent, and the highest percentage, 68, occurred at the end of one hour. The corresponding figures for thin people are 28 per cent with a peak of 75 per cent after half an hour. Normals showed a basal of 20 per cent and 79 per cent after half an hour.

The percentage of calories derived from fat was decreased in all cases after the carbohydrate meal. In some the use of fat was stopped entirely for one hour, and three of the thin people used no fat after three hours. The three normals all ceased using fat for one hour. There was little difference among the three groups in calories derived from protein after a carbohydrate meal. Most of the cases in all groups had a slight decrease from the basal. The averages for the obese group are 29 per cent basal, 24, 23, 18 and 18 per cent in the following periods, figures for the underweights are 25 per cent basal, followed by 22, 27, 21 and 19 per cent, respectively, those corresponding for normals are 24, 21, 25, 18 and 18 per cent. The continued low use of protein for energy after the carbohydrate meal can be accounted for by the sparing effect of carbohydrate on protein, which is a well established fact.

As might be expected, after the ingestion of a high carbohydrate meal, most of the calories expended as energy were derived from carbohydrate. Even at the end of three hours, most of the subjects were still deriving much more energy from carbohydrate than during the basal period, but still considerably less than at the peak of the curve. This holds for all groups of subjects.

Tables 7, 8 and 9 are concerned with the high fat meal. Calories obtained from carbohydrate after the ingestion of a high fat meal are in most cases decreased from the basal until the end of the eight hour period. The overweight people, on the whole, used more carbohydrate than those of the other two groups, having an average of 37 per cent for a basal, 24, 28, 22 and 11 per cent at the end of each of the following

TABLE 4—Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Carbohydrate Meal
Calories from Carbohydrate

Name	Sex	Age	Height, Cm	Weight		Carbo- hydrate Intake, Gm	Heat Production											
				Kilo grams	Percentage of Difference from Normal		Basal		½ Hour after Meal		1 Hour after Meal		2 Hours after Meal		3 Hours after Meal			
							Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total
A D	♂	16	169.5	82.4	+33	104.3	17	23	49	60	42	54	68	80	36	48		
M S	♀	33	159.5	140.0	+141	103.4	28	32	79	73	75	76	71	70	67	72		
S A	♀	25	149.5	80.0	+51	106.4	4	6	44	56	43	58	47	64	48	67		
E S	♀	34	165.0	117.9	+90	80.2	1	1	13	13			25	25	13	14		
E S	♀	28	165.0	83.6	+39	100.0	13	20	62	75	47	63	62	84	51	74		
E S	♀	28	165.0	84.1	+40	100.0	4	6	58	77	43	59	46	67	32	45		
F S	♀	28	165.0	85.5	+42	100.0	14	18	58	72	53	69	36	50	38	54		
E S	♀	28	165.0	86.3	+44	100.0	19	27	65	78	54	73	51	69	47	67		
R K	♀	26	148.5	78.3	+48	106.2	22	27	36	46	57	65	40	53	26	36		
E M	♀	13	161.5	100.0	+102	101.5	26	31	79	78	83	83	40	47	32	40		
E P	♀	34	163.8	101.2	+68	103.4	22	32	74	84	66	81	42	55	16	21		
Average		27	161.6	94.5	+63	100.8	15	20	56	65	56	68	48	60	37	49		
Underweight																		
S S	♂	40	174.0	51.0	-30	100.0	13	21	41	62	39	59	44	66	31	48		
K B	♀	25	182.0	61.4	-20	100.0	26	41	62	89			50	69	34	55		
D	♀	21	158.8	41.6	-26	107.0	18	37	47	82	47	78	54	89	53	90		
E T	♀	22	162.6	51.2	-11	102.9	13	27	43	76	44	78	46	79	16	33		
J W	♀	35	172.0	61.0	-11	103.2	23	40	52	75	49	66	50	76	19	36		
H B	♀	19	167.6	50.8	-14	105.2	9	16	38	65	31	55	29	55	28	56		
M C	♀	24	160.0	44.4	-21	105.4	8	15	50	79	49	77	52	91	32	61		
Average		27	168.1	51.1	-19	103.5	16	28	48	75	43	69	46	74	30	54		
Normal																		
M W	♀	24	160.0	55.5	-1	104.9	24	39	55	77	52	73	45	65	53	83		
D K	♀	22	163.5	58.2	-1	104.4	0	0	54	77	48	70	36	55	33	56		
J S	♀	21	161.3	58.2	+3	106.0	12	20	64	83	61	82	36	55	7	12		
Average		22	161.6	57.3	+0.3	105.0	12	20	58	79	54	78	39	58	31	50		

TABLE 5—Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Carbohydrate Meal
Calories from Fat

Name	Sex	Age	Height, Cm	Weight		Carbo- hydrate Intake, Gm	Heat Production			1 Hour after Meal			2 Hours after Meal			3 Hours after Meal		
				Kilo- grams	Percentage of Difference from Normal		Basal		½ Hour after Meal		Calories per Hour		Calories per Hour		Calories per Hour		Calories per Hour	
							Calories per Hour	Per centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total
							Overweight											
A D	♂	16	169.5	82.1	+ 33	104.3	43	50	13	16	18	23	0	0	14	19		
M S	♂	23	169.5	140.0	+ 141	103.4	44	50	0	0	9	9	5	5	14	15		
S A	♂	25	149.5	80.0	+ 51	106.4	48	67	11	14	16	22	15	21	15	21		
L S	♂	34	165.0	117.8	+ 90	80.2	77	81	76	74	7	9	64	65	68	73		
E S	♂	28	165.0	83.6	+ 39	100.0	21	33	0	0	7	9	2	3	10	16		
F S	♂	28	165.0	84.1	+ 40	100.0	46	65	0	0	7	9	9	13	29	41		
I S	♂	28	165.0	85.5	+ 42	100.0	35	46	0	0	0	0	21	29	20	29		
E S	♂	28	165.0	86.3	+ 44	100.0	31	43	0	0	0	0	11	15	12	17		
E R	♂	26	148.5	78.3	+ 48	106.2	31	78	13	17	14	16	18	24	30	41		
K M	♂	13	161.5	100.0	+ 102	104.5	55	12	0	0	0	0	28	33	34	43		
L P	♂	34	163.8	101.2	+ 68	103.4	27	39	0	0	0	0	31	27	47	61		
Average		27	161.6	91.5	+ 63	100.8	10	51	10	11	7	9	18	21	27	34		
							Underweight											
S S	♂	40	174.0	51.0	- 30	100.0	31	52	10	15	6	9	8	12	21	33		
K B	♂	25	182.0	61.4	- 20	100.0	21	33	2	3	0	0	0	0	18	29		
D	♂	21	158.8	41.6	- 26	107.0	22	16	0	0	0	0	0	0	0	0		
T	♂	22	162.6	51.2	- 11	102.0	23	16	0	0	0	0	0	0	21	45		
J W	♂	35	172.0	61.0	- 11	103.2	17	30	0	0	0	8	0	0	21	39		
H B	♂	19	167.6	50.8	- 14	107.2	32	70	0	0	5	9	13	24	12	28		
M C	♂	24	160.0	44.4	- 21	105.4	31	62	0	0	0	0	0	0	9	18		
Average		27	168.1	51.6	- 19	103.5	26	47	2	3	3	1	3	5	15	27		
							Normal											
M W	♂	24	160.0	55.5	- 1	104.0	19	31	0	0	0	0	11	16	0	0		
D K	♂	22	163.5	58.2	- 1	104.4	42	78	0	0	0	0	19	29	14	24		
J S	♂	21	161.3	53.2	+ 3	106.0	36	60	0	0	0	0	19	29	42	70		
Average		22	161.6	57.3	+ 0.3	105.0	32	56	0	0	0	0	16	25	19	31		

TABLE 6—*Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Carbohydrate Meal*
Calories from Protein

Name	Sex	Age	Height, Cm	Weight		Carbo- hydrate Intake, Gm	Heat Production									
				Kilo grams	Percentage of Difference from Normal		Basal		½ Hour after Meal		1 Hour after Meal		2 Hours after Meal		3 Hours after Meal	
							Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total
A D	♂	16	169.5	82.4	+ 33	104.3	13	18	19	24	18	23	17	20	25	33
M S	♂	33	159.5	140.0	+141	103.4	16	18	29	27	15	15	25	25	15	16
S A	♂	25	149.5	80.0	+ 51	106.4	20	28	23	30	15	20	11	15	9	13
E S	♀	34	165.0	117.8	+ 90	80.2	17	18	13	13	21	28	10	10	12	13
E S	♀	28	165.0	83.6	+ 39	100.0	30	47	21	25	21	28	10	14	8	12
L S	♀	28	165.0	84.1	+ 40	100.0	27	30	17	23	24	31	14	20	10	14
E S	♀	28	165.0	85.5	+ 42	100.0	21	36	23	28	24	31	15	21	12	17
E S	♀	28	165.0	86.3	+ 44	100.0	21	30	18	22	20	27	12	16	11	16
R K	♂	26	148.5	73.3	+ 48	106.2	28	35	29	37	17	19	18	24	23	17
L M	♂	13	161.5	100.0	+102	104.5	22	27	23	23	17	17	17	20	14	18
F P	♀	34	163.8	101.2	+ 68	103.4	20	29	14	16	15	19	14	18	14	18
Average		27	161.6	94.5	+ 63	100.8	21	29	21	24	18	23	15	18	13	18
S S	♂	10	174.0	51.0	-30	100.0	16	27	15	23	21	32	16	24	12	18
K B	♂	25	182.0	61.4	-20	100.0	16	25	5	7			22	31	10	16
D B	♀	21	153.8	41.6	-26	101.0	8	17	10	18	13	22	7	11	6	10
J T	♂	22	162.6	51.2	-11	102.9	13	27	13	24	13	22	12	21	10	22
J W	♂	35	172.0	61.0	-11	103.2	17	30	18	26	19	26	16	24	14	26
H B	♂	19	167.6	50.8	-14	103.2	14	25	20	35	20	36	11	21	10	21
M C	♀	24	160.0	44.4	-21	105.4	12	23	13	21	14	23	12	19	12	22
Average		27	168.1	51.6	-19	103.5	14	25	13	22	17	27	14	21	11	19
M W	♀	24	160.0	53.5	-1	104.9	18	20	16	23	19	27	13	19	11	17
D K	♀	22	163.5	53.2	-1	104.4	12	22	16	23	21	30	11	17	12	20
J S	♀	21	161.3	53.2	+ 3	103.0	12	20	13	17	13	18	11	17	11	18
Average		22	161.6	57.3	+0.3	105.0	14	24	15	21	18	25	12	18	11	18

two hour periods. Corresponding averages for underweight people are 18, 17, 16, 14 and 13 per cent, and for normal 17, 21, 14, 9 and 7 per cent. Here again the obese people seem to tend to use their carbohydrate for energy and to store the fat, but the normal and thin people use up the fat ingested to produce heat.

All groups showed an increase in energy derived from fat after the ingestion of a high fat meal. The greatest increase, however, is found

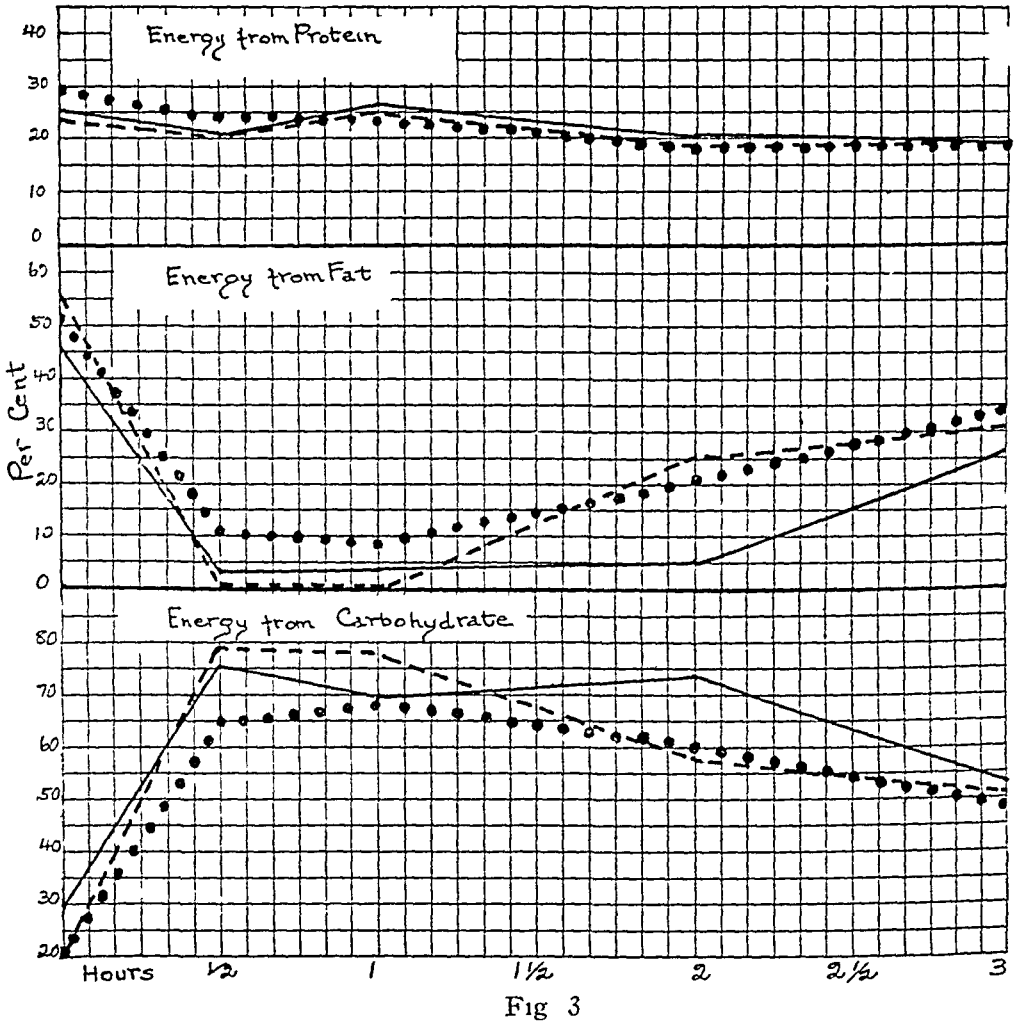


Fig 3

Chart 3—Average percentage distribution of energy production after a high carbohydrate meal. Solid dot—overweight, solid line—underweight, and broken line—normal.

in the thin and in the normal groups. Average basal values for the obese are 46 per cent, followed by 60, 49, 59 and 71 per cent after each two hour period, for underweights 55, 60, 64, 68 and 75 per cent, and corresponding figures for normals, 66, 61, 71, 77 and 79 per cent.

Energy derived from protein is very little affected by the ingestion of a high fat meal in the three groups, and especially in the case of the

TABLE 7--Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Fat Meal
Calories from Carbohydrate

Name	Sex	Age	Height, Cm	Weight		Fat Intake, Gm	Heat Production														
				Kilo grams	Percentage of Difference from Normal		Basal			2 Hours after Meal			4 Hours after Meal			6 Hours after Meal			8 Hours after Meal		
							Calories per Hour	Per- centage of Total	Overweight	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total
M S	♀	33	159.5	131.5	+136	54.7	40	41	Overweight	22	23	23	23	23	28	29	1	1			
A D	♂	16	169.5	83.7	+35	109.0	28	39		19	24	26	26	32	16	15	21	18			
A. D	♂	16	169.5	87.7	+46	82.8	25	32		20	24	24	24	29	14	13	17	15			
E M	♀	13	161.5	98.6	+99	129.1	12	14		12	15	11	11	13	1	6	1	8			
Average		19	165.0	100.3	+79	93.9	26	37		18	24	21	21	28	15	22	9	11			
B D	♀	21	159.1	41.5	-26	50.7	15	31	Underweight	22	42	19	19	37	18	32	9	17			
S S	♂	42	173.4	50.2	-31	53.3	13	21		22	32	10	10	16	8	5	13	8			
H B	♀	19	167.6	50.7	-15	55.3	4	7		4	7	8	8	15	1	7	13	13			
E T	♀	22	163.1	50.8	-11	76.1	8	15		11	19	13	13	23	9	15	17	27			
M O	♀	24	160.3	44.5	-20	39.9	13	25		1	2	0	0	0	10	6	17	11			
J W	♀	35	172.0	61.7	-10	78.9	6	10		0	0	3	3	5	3	2	5	3			
Average		27	166.1	49.9	-19	59.0	10	18		10	17	9	9	16	7	14	7	13			
M W	♀	24	160.0	55.0	-2	79.8	18	30	Normal	13	20	17	17	27	8	12	14	22			
D K	♀	22	163.5	58.2	+2	84.6	11	19		27	44	6	6	10	2	0	3	0			
J S	♀	21	160.3	57.2	+2	55.0	1	2		0	0	3	3	5	7	0	11	0			
Average		22	161.3	56.8	+1	73.1	10	17		13	21	9	9	14	6	9	5	7			

TABLE 8.—*Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Fat Meal*
Calories from Fat

Name	Sex	Age	Weight		Heat Production																
			Height, Cm	Kilo grams	Percentage of Difference from Normal	Fat Intake, Gm	Basal			2 Hours after Meal			4 Hours after Meal			6 Hours after Meal			8 Hours after Meal		
							Calories per Hour	Per- centage of Total	Overweight	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total
M S	♀	33	159.5	131.3	+136	54.7	47	48	Overweight	64	67	57	57	52	54	52	54	75	83		
A D	♂	16	169.5	83.7	+35	109.0	29	41		42	53	35	44	48	62	48	62	52	63		
A D	♂	16	169.5	87.7	+46	82.8	39	50		49	60	38	46	51	61	51	61	58	68		
E M	♀	13	161.5	93.6	+99	129.1	55	65		52	63	64	77	72	87	66	87	66	80		
Average		19	165.0	100.3	+79	93.9	43	46		52	60	49	49	56	59	56	59	63	71		
B D	♂	21	159.1	41.5	-26	50.7	20	41	Underweight	16	30	23	45	28	50	28	50	38	72		
S S	♂	42	173.4	50.2	-31	53.3	34	56		31	46	38	60	13	67	13	67	50	76		
H B	♂	19	167.6	50.7	-15	55.3	34	64		37	68	35	63	44	81	44	81	41	72		
E T	♀	22	163.1	50.8	-11	76.1	32	60		32	57	32	57	33	63	33	63	31	54		
M C	♀	24	160.3	44.5	-20	39.9	27	53		43	78	46	81	41	70	41	70	49	81		
J W	♀	35	172.0	61.7	-10	78.9	32	58	19	79	51	79	51	80	51	80	53	94			
Average		27	166.4	49.9	-19	59.0	30	55	35	60	37	64	10	68	10	68	41	75			
M W	♂	24	160.0	55.0	-2	79.8	29	48	Normal	39	61	36	58	48	73	48	73	42	65		
D K	♂	22	163.5	53.2	+2	81.5	38	65		22	36	44	72	47	78	47	78	45	79		
J S	♀	21	160.3	57.2	+2	55.0	51	85		55	85	52	84	52	81	52	81	53	93		
Average		22	161.3	56.8	+1	73.1	39	66	39	61	14	71	19	77	19	77	47	79			

TABLE 9—Distribution of Total Energy Between Protein, Fat and Carbohydrate After High Fat Meal
Calories from Protein

Name	Sex	Age	Height, Cm	Weight		Fat Intake, Gm	Heat Production													
				Kilo- grams	Percentage of Difference from Normal		Basal		2 Hours after Meal			4 Hours after Meal			6 Hours after Meal			8 Hours after Meal		
							Calories per Hour	Per centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total	Calories per Hour	Per- centage of Total		
M S	♀	33	159.5	131.3	+136	54.7	10	10	10	10	21	21	17	14	14	16				
A D	♂	16	169.5	83.7	+ 35	109.0	14	20	18	23	19	23	13	15	15	18				
A D	♂	16	169.5	87.7	+ 46	82.8	14	18	13	16	21	26	18	22	14	16				
E M	♀	13	161.5	98.6	+ 99	129.1	18	21	18	22	8	10	10	12	10	12				
Average		19	165.0	100.3	+ 79	93.9	14	17	15	18	17	20	15	17	13	16				
B D	♀	21	159.1	41.5	-26	50.7	14	29	15	28	9	18	10	18	6	11				
S S	♀	22	173.4	50.2	-31	53.3	14	23	15	22	15	24	14	22	11	17				
H B	♀	19	167.6	50.7	-15	55.3	16	29	14	25	13	23	9	17	9	15				
I T	♀	22	163.1	50.8	-11	76.1	13	25	14	24	11	20	11	21	11	19				
M C	♀	24	160.3	44.5	-20	39.9	11	22	11	20	11	19	8	13	5	8				
J W	♀	35	172.0	61.7	-10	78.9	17	31	13	21	10	16	5	8	2	3				
Average		27	166.4	49.9	-19	59.0	14	27	14	23	11	20	9	17	7	12				
M W	♀	24	160.0	55.0	-2	79.8	14	23	Normal								9	14		
D K	♀	22	163.5	58.2	+2	84.5	9	16	12	19	9	15	10	15	12	21				
J S	♀	21	160.3	57.2	+2	55.0	8	13	10	20	7	11	11	8	4	7				
Average		22	161.3	56.8	+1	73.1	10	17	11	18	9	15	9	14	8	14				

TABLE 10.—The Respiratory Quotient of Obese, Thin and Normal People After the Ingestion of Protein, Carbohydrate and Fat

Subject	Percentage of Variation from Normal Weight	Respiratory Quotient											
		1*			2			3			4		
		Protein	Carbo- hydrate	Fat	Protein	Carbo- hydrate	Fat	Protein	Carbo- hydrate	Fat	Protein	Carbo- hydrate	Fat
					Overweight								
M N	+72	0.75			0.79			0.80			0.76		
S A	+50	0.808			0.883			0.805			0.782		
	+51		0.754			0.899			0.894	0.908		0.914	
E S	+91	0.732			0.806			0.743			0.738		
	+90		0.725			0.758				0.794		0.762	
A D	+37	0.836			0.888			0.923			0.855		
	+33		0.782			0.908			0.884	0.973		0.877	
	+35			0.837			0.803			0.833			0.789
	+46			0.817			0.793			0.815			0.772
R K	+45	0.80			0.81			0.83			0.80		
	+43	0.772			0.775			0.776			0.789		
	+48		0.819			0.874			0.919	0.800		0.830	
P	+58	0.680			0.773			0.770			0.737		
S	+42	0.78			0.80			0.79			0.76		
	+39		0.81			0.98			0.91	0.97		0.93	
	+40		0.76			0.97			0.91	0.88		0.85	
	+42		0.80			1.02			0.97	0.87		0.88	
	+41		0.82						0.96	0.92		0.91	
F P	+64	0.838			0.831			0.793			0.818		
	+68		0.829			0.971			0.980	0.878		0.783	
E M	+96	0.880†			0.840			0.786			0.747		
	+97				0.853			0.854			0.753		
	+96		0.825			1.040			0.976	0.897		0.839	
	+90			0.802			0.774				0.722		0.744
M S	+141		0.817			0.968			0.945	0.934		0.924	
	+136			0.852			0.777				0.812		0.722

[illegible]

* Periods 1, 2, 3 and 4 in the case of protein and fat occurred two, four, six and eight hours after the ingestion of the meal, in the case of carbohydrate one-half, one, two and three hours after ingestion.

† This subject probably ate something before reporting for the experiment

TABLE 11—Pulse Rate and Heat Production* of Obese, Thin and Normal People After the Ingestion of Protein, Carbohydrate and Fat

Subject	Percentage of Variation from Normal Weight	Basal						1†						2						3						4					
		Pro- tein		Carbo- hydrate		Fat		Pro- tein		Carbo- hydrate		Fat		Pro- tein		Carbo- hydrate		Fat		Pro- tein		Carbo- hydrate		Fat		Pro- tein		Carbo- hydrate		Fat	
		Pulse Rate	Calories	Pulse Rate	Calories*	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories	Pulse Rate	Calories
Overweight																															
M S	+111	70	381	76	424			77	466	85	416			80	427	104	440			78	436	80	420			74	415	75	391		
E M	+96	80	411			94	419							74	418					74	392					74	397				
	+97	75				80	438							79	431					77	399					72	403				
	+96							85	504					87	493																
	+99	80	410	79	420					75	404									82	421	79	409			77	396	74	402		
E S	+91	99	479					90	458					80	416					80	442					80	439				
	+90									80	465									80	412	71	417					79	419		
M N	+72	71	30			82	24							79	23					80	23					82	32				
F P	+64	72	388			64	366							68	391					66	381					62	415				
	+63					58	337			70	425			70	395											86	372				
P	+58	86	335					75	312											83	341					84	333				
S A	+56	71	369			71	425			90	418			73	389					80	376					76	358				
	+51					89	412													88	416					88	415				
R K	+45	90	42			85	47			85	47			87	43					88	43					88	45				
	+43	81	359			80	394			80	394			81	415					76	392					85	374				
	+48					82	469			81	452			79	512											78	422				
A D	+37							84	437					81	464					74	429					74	433				
	+33	78	380					82	418					78	405											78	386				
	+35					76	365																								
	+41					74	394																								
Average		386	394	401		399	454	411		394	413	423		381	425	412				392	404					392	404				

E T		Underweight											
		72 32	68 35	82 36	68 37	80 37	72 37	88 38	72 35	66 30	72 37	66 30	72 37
J W	-11	64 37		66 41		72 41		64 35		64 32		64 32	
	-11				72 36		68 37	72 39		60 32		60 32	
	-10		66 32						64 34				60 33
H B	-14	80 35		76 40		72 10		76 38		68 35		76 32	
	-14		72 34						72 35				80 36
	-15												
Z O	-19	77 36		80 41		81 43		84 39		87 38			
M O	-21												
	-20	84 38	36	86 44	76 39	96 44	84 40	94 45	88 41			88 37	96 43
A K	-20			39				10				34	
M A	-22	90 31		84 39		84 38		82 35		85 32			
	-22	86 33		80 36		84 38		84 35		80 35			
	-26	81 38.2		75.42.1		77 42.6		78 44.8		87 43.8			
B D	-26												
	-26	77 35	76 35	88 41	68 38	93 43	68 37	96 44	80 41	92 43			77 39
B M	-28	85 36		91 45		89 46		89 40		89 41			
S S	-31	55 35.9		62 46.3		64 46.4		74 41.7		64 42.0			
	-30		37		67 43		73 40	42	70 40			11	70 41
	-31												
Average		35.3	35.0	41.3	39.9	41.9	37.8	38.9	40.3	37.4	37.7	35.6	38.2
		Normal											
M W	+1	71 42		73 49		67 46		66 43	81 44	68 41		78 41	
	-1		63 39		68 41		69 40		70 43				64 41
D K	+0	78 36		84 43		83 44		82 42		82 40			
	-1		74 33	87 43	71 38	90 42	80 37	86 41	76 37	80 36			73 36
J S	+2												
	+3	71 37	69 37	76 43	80 48	78 52	63 39	68 41	78 41	69 39	71 37		
	+3												
M S	+3	63 34		68 41	66 41				61 40				52 36
	+2	70 36		71		68 42		66 40		78 39			
	+2					68 43		65 36		65 35			
I T	-7	70 38		74 43		73 40		69 34		74 36			
Average		37.2	36.3	43.8	45.7	41.5	38.7	39.8	42.0	38.8		38.0	37.7

* Computed is calories per square meter of body surface per hour

† Periods 1, 2, 3 and 4 in the case of protein and fat occurred two, four, six and eight hours after the ingestion of the meal in the case of carbohydrate, one half, one, two and three hours after ingestion

overweight and the normal The average percentage of total calories derived from protein by the obese during the basal period was 17 per cent, followed in the two hour periods by 18, 20, 17 and 16 per cent, respectively, the normals showed 17, 18, 15, 14 and 14 per cent for the corresponding periods, and the underweights 27, 23, 20, 17 and 12 per cent Again the underweight people seem to derive more energy from protein

Table 10 is a record of the respiratory quotients of all subjects in all periods It was on these figures that the foregoing work was based

The pulse rate was taken at the end of each metabolism test and is recorded in Table 11 There is no close relation between pulse rate and heat produced per square meter of body surface Making the comparison on one subject at different times or on different subjects at comparable times, we fail to note any interdependence of these two factors For instance, M S on a carbohydrate meal showed a maximum pulse rate of 80 one hour after the meal, whereas her maximum metabolism, 47 calories per square meter, occurred thirty minutes after the meal while the pulse rate was 77 The same is true with most of the other cases M N. showed a drop in metabolism after the high protein meal and a rise in pulse rate Among the underweights the lowest metabolism occurred in conjunction with the highest basal pulse rate, in M A

SUMMARY

1 The present work is concerned with the energy derived from the various nutrients by obese, normal and thin people after the ingestion of meals high in protein, carbohydrate and fat, respectively There were twenty-six tests on obese subjects, twenty-one on thin subjects and twelve on normals

2 After the ingestion of protein, obese people have a tendency to derive their energy from carbohydrate, whereas thin and normal people use less carbohydrate than during starvation Calories derived from fat decrease in the obese after the meal, but show little change in the thin and normals The two latter groups, however, show a decided rise in calories derived from protein after the meal, but the protein consumption is little affected in the obese This confirms our previous finding^s that there is very little specific dynamic action of protein in obese people

3 All subjects derive more energy from carbohydrate after the ingestion of a high carbohydrate meal, and less from fat Calories derived from protein after this meal vary little, however, in any group from the starvation figures

4 After the high fat meal all groups show an increase in energy derived from fat. The obese continue to use carbohydrate in greater amounts than the two other groups. The protein consumption shows very little change, especially in the obese and normals.

5 There is no relation between the pulse rate and energy production computed on the basis of heat produced per square meter of body surface.

6 The fact that the obese subjects derive less energy from fat than either the thin or the normals after meals indicates a reason for excessive fat storage.

DISEASES OF THE LIVER

III A COMPARATIVE STUDY OF CERTAIN TESTS FOR HEPATIC FUNCTION IN PATIENTS WITH OBSTRUCTIVE JAUNDICE *

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Jaundice is the condition due to the presence of bile pigment in the blood and is recognized clinically by the resultant staining of the skin, conjunctiva, mucous membranes, blood serum and, as a rule, the urine by the bile pigment

Like albuminuria, jaundice is a symptom and not a disease, and may be encountered in a number of different conditions, the common and essential factor being obstruction to, or failure of, the normal process of excretion of the bile

A mechanical obstruction to the passage of bile through the larger or extrahepatic bile ducts rapidly leads to resorption of pigments and the development of jaundice. The mechanism of this resorption and the pathway through which the absorbed bile enters the general circulation have been studied by many experimenters from the time of Saunders¹ (1803) to that of Bloom² (1923). The experiments of the former served to emphasize the importance of the resorption of the bile pigment through the lymphatic channels and the thoracic duct in the development of jaundice. The latter likewise finds that after ligation of the common bile duct, bile appears in the lymphatic vessels before it can be demonstrated in the blood stream. The obstructive type of jaundice has long been recognized as one of the least complicated forms of icterus. The other varieties of icterus, the so-called hepatogenous and hematogenous forms, will be reviewed in connection with the clinical discussion of toxic or infectious jaundice and the hemolytic anemias.

The clinical symptoms of obstruction to the biliary passages are characteristic and distinct. Icterus, staining of the secretions, especially of the urine with bile pigment, the absence of bile from the stools with

*From the Division of Medicine, Mayo Clinic and the Mayo Foundation, and the Division of Surgery, Mayo Clinic

1 Saunders, William. A Treatise on the Structure, Economy and Diseases of the Liver with an Inquiry into the Properties and Competent Parts of Bile and Biliary Calculi, Ed 4, London Phillips, 1809

2 Bloom, William. The Rôle of the Lymphatics in the Absorption of Bile Pigment from the Liver in Early Obstructive Jaundice, Bull Johns Hopkins Hosp 34 316-320 (Sept) 1923

resultant disturbance in fat absorption, producing the characteristic acholic, clay-colored stool, pruritus, and a delay in the coagulation time of the blood with consequent tendency to hemorrhage are constantly present. Bradycardia is less common. Pain, chills, fever and other evidences of infection, such as leukocytosis, are variable. Gastro-intestinal disturbance, with nausea, anorexia and loss in weight and strength, is common, this is also further aggravated by the primary nutritional disturbance consequent on the absence of bile from the intestine. Cerebral symptoms also occur, but these are usually associated with a terminal toxemia and appear late in the sequence of manifestations.

Jaundice due to obstruction of the biliary passages is well tolerated in the main. Cases have been reported with persistent and possibly continuous icterus for years without serious disturbance of the health of the patient apart from the discoloration of the skin and pruritus. Such a condition is in marked contrast to the icterus gravis associated with acute yellow atrophy of the liver, in which the marked disturbance in the activity of the liver results in the rapid development of profound toxemia and death. Although obstructive jaundice is often well borne and recovery usually rapid following relief from the obstruction, a certain proportion of patients do not stand operation and die with symptoms of toxemia somewhat similar to those observed in cases of acute yellow atrophy.

That obstructive jaundice is not without an effect on the liver is evidenced by the production of the pathologic changes characteristic of biliary cirrhosis by bile retention over any extended period. Physiologic disturbances in the activity of the liver, apart from the jaundice, have not been so conclusively shown to accompany these morphologic changes. As we have indicated in the initial paper of this series,³ this is in part due to the difficulty of obtaining exact criteria and reliable quantitative tests for disturbances in the activity of the liver. We have been able to show disturbances of hepatic function in animals following the experimental ligation of the common bile duct,⁴ in which a parallel increase in the serum bilirubin and degree of retention of phenoltetrachlorophthalein was the most striking change noted. We shall present here the results of the study of a series of cases of obstructive jaundice.

The cases may be divided into two groups, the larger comprising forty-three cases of obstructive jaundice, the smaller, twenty-four cases of chronic cholecystitis without clinical evidences of jaundice. The latter

3 Greene, C. H., Snell, A. M., and Walters, Waltman. Clinical and Experimental Studies in Diseases of the Liver, I, A Survey of Tests for Hepatic Function, *Arch. Int. Med.*, to be published.

4 Snell, A. M., Greene, C. H., and Rowntree, L. G. Clinical and Experimental Studies in Diseases of the Liver, II, A Comparative Study of Certain Tests for Hepatic Function in Experimental Obstructive Jaundice, *Arch. Int. Med.*, to be published.

group serves as a control of the effect of cholecystic disease alone. The technic of the laboratory tests and the normal values for each have been reported in the first paper of the series.³

OBSTRUCTIVE JAUNDICE

Obstructive Jaundice Due to Stone in the Common Bile Duct—Cabot⁵ reports that the most common types of jaundice are the icterus neonatorum and that which owes its origin to an infection or sepsis. Jaundice due to stone in the common duct is the next in order of frequency. In Table 1 are shown the different laboratory findings in a series of cases of obstructive jaundice due to the impaction of stone in the common bile duct. In each case the diagnosis was verified by the surgeon's findings at operation. The complete history in each case is not essential to this study, but the histories of two illustrative cases are included to show the general nature of the clinical findings.

Only those positive findings on which conclusions are based at the present time are included in the case reports. The phenoltetrachlorophthalein reading is recorded as for the end of the first hour only. Complete findings are included in the tables. Complete details of the surgical procedure are not given, since the patients were regularly prepared according to Walters'⁶ method.

REPORT OF CASES

CASE 1 (Case 12, Table 1)—*Chronic cholecystitis with cholelithiasis, cholecholelithiasis and obstructive jaundice*—A man, aged 59, came to the Mayo Clinic, June 17, 1924, because of jaundice of five weeks' duration. Eighteen months before, he had had an attack of severe pain at the right costal margin which radiated to the shoulder. Five weeks before, he had had a second similar attack, three days after which the stools became acholic, the urine saffron, and persistent jaundice developed. The patient lost 20 pounds (9 kg) in the five weeks before coming to the clinic. Marked jaundice was present, but the liver was not palpable. The serum bilirubin was 182 mg. There was marked retention of phenoltetrachlorophthalein, the reading being 30 per cent. Chronic cholecystitis and cholelithiasis were found, and there was a stone in the ampulla of Vater. There were signs of chronic pancreatitis and chronic hepatitis. The gallbladder and the stones were removed and a drain placed in the common duct. Bile drained freely. Eighteen days after operation, the serum bilirubin had fallen to 67 mg and the phenoltetrachlorophthalein retention to 8 per cent.

CASE 2 (Case 14, Table 1, Fig 1)—*Chronic cholecystitis with cholelithiasis and obstructive jaundice*—A man, aged 63, came to the Mayo Clinic, June 16, 1924, complaining of abdominal pain and jaundice of six weeks' duration. For twenty-five years he had had bilious attacks associated with nausea, and pain in the right upper quadrant. He had been definitely jaundiced once, four or five years before. There had been acute attacks recently with severe epigastric pain radiating to the right shoulder, and after the last attack the urine became dark and the stools acholic, accompanied by intense pruritus and jaundice.

5 Cabot, R. C. *Differential Diagnosis*, Ed 2, Philadelphia, W. B. Saunders Company, 1912.

6 Walters, W. *Preoperative Preparation of Patients with Obstructive Jaundice*, Surg., Gynec. & Obst. **33** 651 (Dec.) 1921.

The patient lost 35 pounds (15.9 kg) in the preceding six months. The liver edge was palpable, but the gallbladder was not. Roentgenograms of the gallbladder area showed stones. The serum bilirubin was 16 mg, the phenoltetrachlorophthalein reading of 28 per cent indicated marked retention. At operation the liver was found to be hard and congested. The gallbladder contained no bile, but was completely adherent to a large gallstone which compressed the common duct. Cholecystectomy was performed and a drain inserted in the common duct.

Biliary drainage was established but failed progressively, while the jaundice increased and a marked toxemia developed. Four days after operation the patient was critically ill, there was no bile drainage, the serum bilirubin was 169 mg and the blood urea 146 mg. The intravenous administration of 500 cc of 20 per cent glucose solution produced a marked improvement in the general clinical condition. The bile flow was reestablished. Three days later the blood urea was 111 mg and the serum bilirubin 67 mg. Improvement continued.

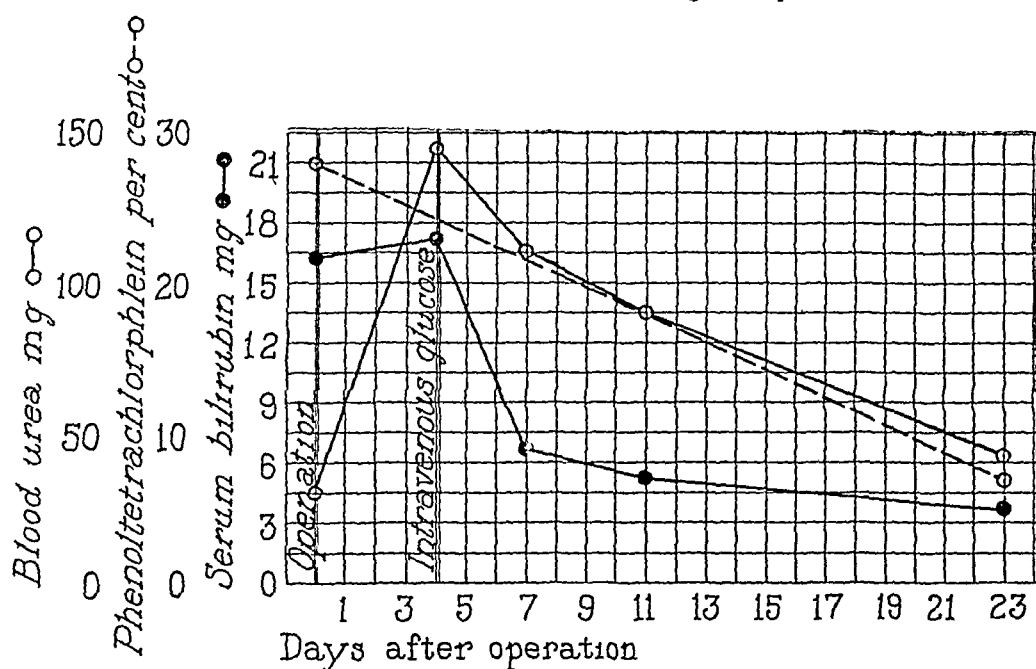


Fig 1—Changes in serum bilirubin, phenoltetrachlorophthalein retention and blood urea in Case 2

and the patient was dismissed about three weeks after the operation. At that time the serum bilirubin was 38 mg, the phenoltetrachlorophthalein retention was 7 per cent, and the blood urea 42 mg.

Obstructive Jaundice Due to Postoperative Structure of the Common Duct—Present surgical practice is to make cholecystectomy the operation of choice in the majority of cases of gallbladder disease. Injury to the ducts during the removal of the gallbladder is an accident dreaded by every surgeon. Judd and Burden⁷ have recently pointed out the factors that predispose to damage. If the injury is recognized immediately, repair usually can be easily and satisfactorily made. When the injury escapes the attention of the surgeon, nature proceeds with repair, but

7 Judd, E. S., and Burden, V. G. Postoperative Structure of the Common Bile Duct, *Ann Surg* 80:210-216 (Aug) 1924

ABLE 1—Obstructive

Case	Date	Age, Years	Sex*	Edge of Liver Pal- pable, Cm	Jaundice	Duration of Jaundice	Urine				Blood Count			Phenolsulphone phthalein, per Cent	Blood Nitrogen Partition, Mg , per Cent				
							Specific Gravity	Albumin	Casts	Bile Pigments	Hemoglobin, per Cent	Erythrocytes, Millions	Leukocytes		Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen
						Cases with Obstruction of the Common Duct													
1	11/ 5/24	54	♂	0	3	1 wk	1 006	0	0	+	75	4 65	9,800		25	9	36	13	66
2	10/ 7/24	42	♀	0	3	2 mo	1 008	0-1	0	+	64	3 66	7,600	45	21	16	27	15	54
3	5/10/24	49	♀	1	2	10 mo	1 031	0-1	0	+	72	4 12	5,200		28	25	23	14	62
4	7/17/23	45	♀	5	3	12 mo	1 013	0	1		83	4 06	10,000			27	28	20	
5	2/13/24	69	♀	+	1	2 wk	1 016	1	1-3		72	1 10	5,200	45	43	32			
6	5/ 1/24	53	♀	En- larged	3	7 wk	1 005	0-1	1	+	68	3 82	6,400	40	31	31	33	12	61
7	6/ 6/23	67	♂	0	2	Inter- mittent	1 022	1	1	+	70	4 07	6,300	60	28	38	00	16	46
8	9/ 5/23	46	♀	+	3	24 mo	1 005	0-1	0		63	3 70	7,600	50		18	29	12	
9	1/22/24	70	♂	+	4	1 wk	1 021	2	1-4	+	78	4 43	7,500		37	36			
10	4/22/24	60	♀	4	2	3 wk	1 031	1	0	+	73	4 03	6,300	55	29	31	23	13	58
11	6/ 5/23	55	♂	En- larged	2	2 mo	1 010	0	0		76	5 25	8,600			22	31	16	70
Cases Studied Before and After Relief of Obstruction																			
12	6/20/24	59	♂	0	3	1 mo	1 001	0	0		68	3 80	5,400		24	15	00	12	57
	7/17/24			0	1										28	23	22	18	53
13	6/ 2/24	62	♀	3	4	12 mo	1 031	0-1	0	+	60	3 80	6,200		31	55	29	13	61
	6/13/24			3	3		1 017	0	0	+					30	32	21	17	53
14	6/23/24	63	♂	1	4	5 wk	1 012	0-1	1	+	70	4 28	8,000		33	30	34	16	50
	7/17/24			1	1										47	42	41	23	54
15	5/30/24	43	♂	4	3	2 wk	1 027	3	1	+	69	3 93	9,400		34		29	18	61
	6/17/24			4	0										39	33	27	21	53
16	6/ 3/24	59	♂	0	2	6 mo	1 016	0-1	0	+	68	3 85	5,000		19	18	17	17	57
	6/26/24			0	1												23	23	13
17	10/10/23	37	♂	5	0		1 028	2	1	0	70	4 40	10 600	50		35	48	16	

* In this and the following tables, ♂ indicates male, ♀, female

TABLE 2—Obstructive Jaundice

Case	Date	Age, Years	Sex	Edge of Liver Pal- pable, Cm	Jaundice	Duration of Jaundice	Urine				Blood Count			Phenolsulphone phthalein, per Cent	Blood Nitrogen Partition, Mg , per Cent				
							Specific Gravity	Albumin	Casts	Bile Pigments	Hemoglobin, per Cent	Erythrocytes, Millions	Leukocytes		Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen
Cases with Obstruction of the Common Duct																			
18	6/18/24	28	♂	Pal- pable	4	7 mo	1 015	1-2	0	+	86	4 96	10,400	80	24	15	10	13	53
19	9/26/24	47	♀	En- larged	4	6 wk	1 023	1	1	3	67	4 13	5,200		28		23	13	53
20	7/12/23	21	♀	5	3	1 mo	1 016	1	0		80	4 95	5 600	35		20	21	13	
21	10/17/24	31	♀	0	3	9 wk	1 020	1	0	3	74	3 83	9,200	45	24	19	26	13	58
22	11/ 4/24	40	♀	En- larged	3	36 mo	1 022	1	0		27	2 78	7,200		35	31	20	14	
23	3/28/24	45	♀	0	3	11 mo	1 021	0-2	0	+					26	17	18	12	59
24	5/27/24	55	♂	0	4	18 mo	1 018	1	0	2	68	4 38	4,300	50	27	48	24	18	58
25	11/20/24	56	♂	0	1	18 mo	1 011	0-1	0	0	72	4 43	10,100		31	24	33	13	65
26	3/15/24	56	♂	0	2	8 mo	1 022	0	1		79	4 39	8,400		36	30	24	16	68
Cases Studied Before and After Relief of Obstruction																			
27	3/ 7/24	34	♂	4	4	2 wk	1 025	1-2	1	2	63	4 16	9,400	65	27	20			
	3/15/24			4	2											29			
	3/27/24			4	1											23			
28	3/17/24	34	♂	2	4	6 mo	1 021	1-2	2	4	64	3 89	10,900	60	32	12	17	14	64
	4/ 7/24			2	2		1 009	0-1	0	1					26	12	29	11	47
	5/27/24			1	0		1 014	0-1	0	0				65	27	25	36	19	57
	10/25/24			1	2	3 mo	1 023	0-1	0	+					26	27	15		63
	11/17/24			1	0		1 021	0-1	0	0					26	28	16		
29	1/14/24	39	♂	++	1	14 mo	1 016	0-1	0		74	4 94	6,900			15	18	19	
	1/21/24			++	0		1 022	0	0							19			
	3/ 4/24			++	1	1 wk	1 015	0	0						27				
	9/26/24			++	2	6 mo	1 021	0	0		74	4 81	11,100	30	23	11	17	61	
30	6/ 4/23	59	♂	0	0		1 018	0-1	1-2		80	3 75	4,200	60		23	25	14	67
	8/ 8/23			0	0											23	20	17	
31	4/ 5/24	49	♀	0	0		1 014	0	0	0	70	4 08	7,600		26	18	20	08	56
32	3/10/24	55	♀	Pal- pable	1	21 mo	1 022	0-1	0	0	68	3 86	9,100		27	20			

Bile Index	Bile Pigments (Serum)		Coagulation Time		Fructose Tolerance, Blood Sugar, Mg, per Cent				Phenoltetrachlor phthalein				Comment	
	Serum Bilirubin, Mg, per Cent	Van den Bergh Direct Reaction	Minutes	Seconds	Normal	1 Hour	2 Hours	Change	Dye in Serum, per Cent			Dye in Urine, Mg		
									15 Minutes	1 Hour	2 Hours			
Cases with Obstruction of the Common Duct														
85	15.6	+	7	45						34	26	2.2	Chronic cholecystitis with stones, choledocholithiasis	
	5.5	+	10	30						30	26		Chronic cholecystitis with stones, choledocholithiasis	
36	9.0	+	9	30	102	122	113	23	23	30	23	1.0	Chronic cholecystitis, stone in hepatic duct	
45			5	14					27	25	25	4.0	Chronic cholecystitis, choledocholithiasis	
20	4.4	+	8	50	95	138	133	43	30	25	20	1.9	Choledocholithiasis, pancreatitis	
48	11.2	+	8	30	94	114	127	33	23	25	20	3.6	Chronic cholecystitis, choledocholithiasis	
50	10.9	+	8	0	87	110	121	34	18	24	22	6.0	Chronic cholecystitis with stones	
			9	5					20	20	20	5.2	Died, choledocholithiasis, cholecystoduodenal fistula	
43	8.1	+	7	20	110	138	127	28	27	18	15	1.5	Choledocholithiasis, biliary cirrhosis	
42	6.0	+								13	8	0.5	Chronic cholecystitis, obstructive jaundice	
20			5	25					13	12	6	1.0	Cholelithiasis, obstructive jaundice	
Cases Studied Before and After Relief of Obstruction														
	18.2	+	9	20	110	130	106	20	30	30	30	5.8	Choledocholithiasis, pancreatitis	
	6.7	+			75	96	78	21	10	8	6	1.1	18 days postoperative	
75	20.3	+			149	158	177	28	30	30	30	1.8	Choledocholithiasis, obesity, diabetes	
48	7.6	+	7	20	185	244	219	59	18	22	22	1.4	After decrease in jaundice	
75	16.2	+	7	0	87	84	87	-3	24	28	32	2.5	Chronic cholecystitis with stone, obstructive jaundice	
21	3.8	+			97	108	100	11	8	7	3	0.6	3 weeks postoperative	
	18.3	+	8	45	100	140	115	40	23	28	24	0.6	Chronic cholecystitis with stones, choledocholithiasis	
	2.1	+			117		100	-17			2	0.2	15 days postoperative	
	13.4	+	13	0	87	117	91	30	18	22	9	0.7	Choledocholithiasis, cholecystoduodenal fistula	
13	2.5	+			96	104	95	8	16	6	4	0.5	21 days postoperative	
			6	30					25	20	18	0.5	Choledocholithiasis, biliary cirrhosis 3 months after relief of obstruction	

Due to Postoperative Stricture

Bile Index	Bile Pigments (Serum)		Coagulation Time		Fructose Tolerance, Blood Sugar, Mg, per Cent				Phenoltetrachlor phthalein				Comment	
	Serum Bilirubin, Mg, per Cent	Van den Bergh Direct Reaction	Minutes	Seconds	Normal	1 Hour	2 Hours	Change	Dye in Serum, per Cent			Dye in Urine, Mg		
									15 Minutes	1 Hour	2 Hours			
Cases with Obstruction of the Common Duct														
346	52.7	+			100	92	99	-8	30	44	34	8.8	Died, postoperative stricture of common duct, biliary cirrhosis	
	21.1	+	9	30						36	30	7.2	Postoperative stricture of common duct	
					91					30	27	8.2	Postoperative stricture of common duct	
59	10.3	+	8	30						26	26	4.8	Postoperative stricture of common duct	
55	8.8	+	11	0						26	26	5.8	Postoperative stricture of common duct	
34	7.6	+			100	123	110	23		26	24	1.7	Postoperative stricture of common duct	
210	32.5	+	10	30	83	107	87	24	25	25	25	7.8	Postoperative stricture of common duct	
	2.9	+	8	0					14	22	14		Postoperative stricture of common duct with intermittent jaundice	
14	3.2	+			113	130	120	17	22	20	17	1.3	Postoperative stricture of common duct with intermittent jaundice	
Cases Studied Before and After Relief of Obstruction														
77	18.7	+	12	25	120	123	125	5	30	48	44	5.3	Postoperative stricture of common duct	
71	17.2	+							24	24	22		Obstructive jaundice 5 days postoperative	
32	8.7	+			85	90	81	5	25	28	23	3.7	17 days postoperative	
95	24.8	+	19	15	85	105	90	20	30	30	30	7.0	Postoperative stricture of common duct	
26	5.3	+	7	50					9	7	4	1.2	Obstructive jaundice 12 days postoperative	
25	2.5	+	7	20	95	120	93	25	10	10	5	0.9	65 days postoperative	
33	6.3	+			88	100	95	12	15	15	17	0.6	Recurring stricture with obstruction	
	3.4	+			91					9		1.5	15 days postoperative	
			6	30	95	118	92	23	7	3	0	0.3	Postoperative stricture of common duct residual jaundice only	
			10	35						2	1	0	0.0	No jaundice 1 week later
16	3.3	+	9	25					8	11	8		Slight jaundice of 1 week's duration	
	7.6	+			98	104	94	6	26	25	25	2.5	Postoperative stricture with nearly complete obstruction	
									11	10	8	0.2	Postoperative stricture, biliary fistula with partial obstruction	
									2	1	0	0.3	After complete drainage	
7	2.5	+	9	25	85	123	100	38	15	17	12		Postoperative stricture of common duct, complete biliary fistula 5 months	
8	1.5	+	13	0	91	111	123	32	8	7	3	0.5	Postoperative stricture of common duct, partial slight jaundice	

the handicap may be too great and stricture is then prone to follow. Postoperative stricture of the common duct is usually the result of operative trauma, but it may also follow localized infection or necrosis of the wall of the duct. Table 2 shows the laboratory findings in a series of cases of obstructive jaundice originating in this way. The histories of two illustrative cases are also reported in abstract. This report deals with selected cases and the numbers reported in each group should not be interpreted as representing the relative frequency of occurrence of the condition.

CASE 3 (Case 28, Table 2)—*Postoperative stricture of the common duct with obstructive jaundice*—A man, aged 34, came to the Mayo Clinic, March 8, 1924. He had been ill since 1912, and several operations had been performed.

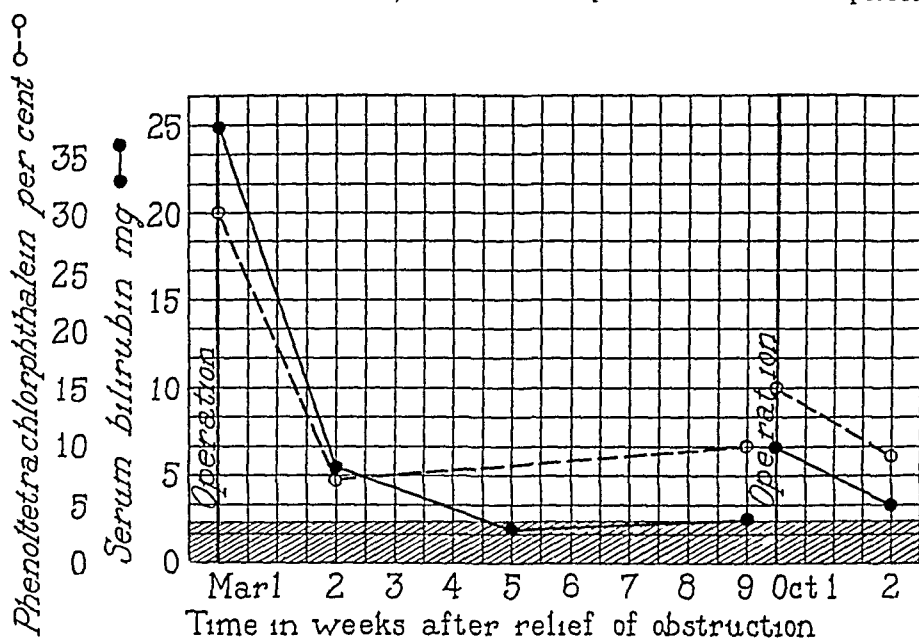


Fig 2—Changes in serum bilirubin and phenoltetrachlorophthalein retention in Case 3. The shaded area indicates the range of the normal values of the two tests. The effects of the relief of the obstruction and of a second postoperative stricture are well shown.

elsewhere without permanent relief. In December, 1922, he began to have intermittent attacks of pain at the right costal margin, radiating to the right shoulder. In March, 1923, the gallbladder was removed. Jaundice appeared the following day, although there was free drainage of bile, but recovery was apparently complete after six or seven weeks. In September he developed persistent jaundice without pain. On admission eight months later, the jaundice was marked. The patient had lost 27 pounds (12.2 kg) during the preceding year. The liver was palpable, the urine contained a trace of albumin and a few granular casts, as well as much bile. The serum bilirubin was 24.8 mg and there was marked retention of phenoltetrachlorophthalein, as indicated by a reading of 30 per cent.

Operation revealed extensive adhesions and obstruction of the common bile duct due to a stricture. Drainage was established, and the reconstruction of the common duct attempted. The postoperative progress was good. Twelve days after the operation, the serum bilirubin was 5.3 mg, while the phenoltetrachlorophthalein showed slight retention with a reading of 7 per cent. The patient was dismissed April 27, 1924, at which time the serum bilirubin was 2.5 mg and the phenoltetrachlorophthalein was 10 per cent.

The patient again became ill June 18, 1924, and jaundice reappeared. It varied in intensity, but was associated at times with pruritus, chills, diarrhea and loss of weight. The patient was readmitted October 25, when the condition was similar to that noted on his first admission. Serum bilirubin was 63 mg and the retention of phenoltetrachlorophthalein was 15 per cent. At operation it was found that the hepatic duct was distorted and obstructed by adhesions. A hepaticoduodenostomy over a rubber tube was made. The operation was difficult, but the postoperative recovery was uneventful. Fifteen days after the operation the jaundice had disappeared, and the serum bilirubin was 34 mg while the dye showed 9 per cent retention. The patient was dismissed a week later.

This case is of particular interest in that it was possible to follow the changes over an extended period. During this time a second stricture developed so that the studies comprise the whole cycle of changes associated with such obstruction.

CASE 4 (Case 29, Table 2) —*Recurring postoperative stricture of the common duct with obstructive jaundice*—A man, aged 39, was admitted to the Mayo Clinic, Jan 14, 1924, complaining of intermittent jaundice during the last two years, initiated by an attack of acute illness with severe pain in the right upper quadrant. A cholecystectomy was performed at the time of the original attack, but drainage of bile persisted for four months. The last attack began in December, 1923, and continued until the time of admission. The patient lost 17 pounds (7.7 kg) during the year. The liver was markedly enlarged, but the jaundice was slight and rapidly disappeared. There was slight retention of phenoltetrachlorophthalein, with the complete disappearance of the jaundice the reading decreased from 3 to 1 per cent. At operation many dense adhesions were found, and the common duct contained stones and gravel. Bile flowed freely from the hepatic duct, and hepaticoduodenostomy was performed. The patient did well for a month, when he had a chill followed by slight jaundice. These attacks recurred and he was readmitted March 4, one week after the last attack. At this time jaundice was slight. The serum bilirubin was 33 mg and the phenoltetrachlorophthalein retention was 11 per cent. Duodenal drainage established a good flow of bile. Progress was satisfactory for a short time, but the attacks of chills, fever and jaundice recurred. The patient was again admitted to the clinic September 27, with marked jaundice. The serum bilirubin was 76 mg and the phenoltetrachlorophthalein showed retention of 25 per cent. A marked contraction at the opening of the former anastomosis had occurred, this was successfully relieved.

Obstructive Jaundice Due to a Tumor of the Head of the Pancreas—Next to stones, pressure on the common duct by an enlargement of the head of the pancreas is perhaps the most common cause of obstructive jaundice. Table 3 includes a group of cases in which the jaundice was consequent on obstruction by swelling in the head of the pancreas. In all cases the tumor demonstrable at operation was presumably carcinomatous in nature. Removal of specimens for diagnosis in such cases is not always feasible, and the macroscopic differentiation of chronic pancreatitis and malignant disease is difficult. In each case, however, the obstruction to the bile duct and the resultant jaundice were the outstanding clinical features. Cases of jaundice with proved carcinomatous involvement of the liver will be reported later.

TABLE 3—Obstructive Jaundice Due

Case	Date	Age, Years	Sex	Edge of Liver Palpable, Cm	Jaundice	Duration of Jaundice	Urine				Blood Count			Phenolsulphone phthalein, per Cent	Blood Nitrogen Partition, Mg, per Cent				
							Specific Gravity	Albumin	Casts	Bile Pigments	Hemoglobin, per Cent	Erythrocytes, Millions	Leukocytes		Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen
Cases with Obstruction of the Common Duct																			
33	6/25/24	62	♂	5	3	1 mo	1 021	0-1	0	+	70	4 04	6,600	55	30	40	2 9	13	50
34	3/15/24	73	♀	Enlarged	4	2 mo	1 016	2	0	3	70	4 21	6,100	46	41	20	3 7	17	
35	4/18/24	53	♂	Enlarged	4	6 da	1 016	2	2	2	65	3 76	5,200	45	33	39	2 6	12	
36	8/ 1/23	34	♀	1	2	1 mo	1 029	1	1		93	4 30	11,600	60		22			
37	6/ 5/24	56	♂	1	3	16 mo	1 024	4	1	2	75	4 18	8,700	40	30	26	2 2	17	51
38	12/11/24	55	♂	3	3	3 mo	1 020	1	1	3	65	4 20	9,100	65	26	16	1 7	11	
39	8/ 7/23	57	♂	Enlarged	3	1 mo	1 003	1	0	2	73	4 26	7,200		149	62			
Cases Studied Before and After Relief of Obstruction																			
40	4/17/24	57	♂	Palpable	0		1 026	0	0	0	65	3 83	5,500		29	38	2 9	15	64
	5/21/24			Palpable	4	4 wk	1 015	0-1	0	+	55	3 30	6,000		34	41	1 6	14	62
41	5/28/24	52	♂	Enlarged	4	6 wk	1 020	2	0	+	55	3 14	7,300		23	38	2 1	14	46
	7/19/24				0		1 024	1	0	0	73		8,200		27	29	3 0	19	56
42	8/ 2/23	52	♂	Enlarged	4	7 wk	1 012	2	1		70	3 54	11,400		26				
	2/ 9/24				1		1 023	0	1				7,700		40	37	3 0	19	
43	10/27/24	44	♂	0	1		1 025	0	0		72	4 39	7,900		37	36	2 7	16	78
	11/ 8/24			0	0		1 007	0	0						23	30	1 5		

TABLE 4—Chronic Cholecystitis

Case	Date	Age, Years	Sex	Edge of Liver Pal- pable, Cm	Jaundice	Duration of Jaundice	Urine				Blood Count			Phenolsulphone- phthalein, per Cent	Blood Nitrogen Partition, Mg , per Cent				
							Specific Gravity	Albumin	Casts	Bile Pigments	Hemoglobin, per Cent	Erythrocytes, Millions	Leukocytes		Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen
44	5/22/23	43	♀	10	0	*	1 027	0-1	+	56	5 20	12,500							78
45	12/ 7/23	57	♀	0	0	*	1 020	1	1		85	5 10	9,800	44		34			
46	7/ 4/24	32	♀	0	0	0	1 028	0	0	0	73	4 17	5,900		43	53	2 3	18	56
	7/ 8/24			0	0	0	1 023	0-1	0	0					34	43	2 1	18	69
47	10/16/24	38	♀	0	0	0	1 012	0-1	0		55	3 11	9,000	80	21	15	1 7	13	48
48	10/ 6/23	73		0	0	*									24	23	1 5		
49	6/ 4/24	47	♀	0	0	0	1 008	0-1	0				5 400		29	37	0 0	15	67
50	11/24/24	46	♀	0	0	0	1 033	0	0		75	4 96	8 900		25	25	1 9	12	62
51	7/ 6/23	43	♀	0	0	*	1 022	0-1		0	77	4 20	6,500		22	28	2 0		
52	5/28/24	51	♀	0	0	0	1 024	0	0		78		5,300	50	30	34	2 3	17	70
53	5/24/24	59	♀	0	0	0	1 031	0-1	0		79	4 63	8,700	55	26	36	2 1	12	64
54	9/17/23	53	♀	1	0	0	1 009	0			75		7,400		20				
55	6/10/24	60	♀	0	0	0	1 022	0	0		70	4 23	7,600	70	34	46	1 7	13	69
56	2/12/24	44	♀	0	0	0	1 019	0-1						60	31	19			
57	4/24/24	49	♀	1	0	*	1 009	0-1	0		72	4 38	10,100	75	27	9	2 6	14	60
58	7/28/24	56	♀	0	0	0	1 014	0	0		72	4 46	8,100	50	31	36	2 5	15	64
59	6/ 9/24	34	♀	0	0	*	1 024	0-1	0	0	64	3 26		60	35	43	2 6	15	58
60	1/23/24	36	♀	3	0	*									32	13			
61	1/21/24	50	♀	0	0	0	1 021	0	1		80	4 30	9,000	60	33	35			
62	7/23/23	46	♀	0	0	0	1 008	0			79		12,900		27	40	2 0		
63	2/ 1/24	39	♀	1	0	*	1 034	1			78	4 31	8,400		32	32			
64	5/29/24	41	♀	0	0	0	1 030	0-1	0						30	26	1 8	17	54
65	10/10/23	43	♂	0	0	0	1 018	0			75	3 86	7,500	50		26			
66	1/ 5/24	44	♂	0	0	0	1 010	0			78	4 64	6,700	55	25	17			
67	7/10/24	47	♀	1	0	0	1 034	0-1	0	0	74	4 10	6 600	65	26	25	2 3	11	70

~ onal attacks of jaundice

to Tumor of Head of Pancreas

Bile Pigments (Serum)			Coagulation Time		Fructose Tolerance, Blood Sugar, Mg, per Cent				Phenoltetrachlorophthalein				Comment	
Bile Index	Serum Bilirubin, Mg, per Cent	Van den Bergh Direct Reaction	Minutes	Seconds	Normal	1 Hour	2 Hours	Change	Dye in Serum, per Cent			Dye in Urine, Mg		
									15 Minutes	1 Hour	2 Hours			
Cases with Obstruction of the Common Duct														
138	27.8	+			112	135	127	23	40	44	34	Died, carcinoma of head of pancreas, obstructive jaundice		
46	24.1	+	10	45	100					36	28	Died, tumor at head of pancreas, obstructive jaundice		
90	35.7	+	4	30						34	33	30	Died, tumor at head of pancreas, obstructive jaundice	
			9	40						30	30	30	4.2	Died, carcinoma of papilla of Vater, obstructive jaundice
41	8.2	+								25	28	25	3.0	Tumor at head of pancreas, obstructive jaundice
	15.0	+									24	20		Died, carcinoma of head of pancreas, obstructive jaundice
			7	20	95	143	132	48		12	3	0.0	Died carcinoma of pancreas, obstructive jaundice	
Cases Studied Before and After Relief of Obstruction														
10	3.5	±	6	50	170				7	4	0	0.4	Died, carcinoma of head of pancreas, before obstruction	
143	35.6	+	13	15	162	197	201	39	24	24	20	4.2	After obstruction	
108	14.2	+	11		85	88	66	3	30	30	30	1.0	Carcinoma of head of pancreas, obstructive jaundice	
18	1.9	+	8		85	104	90	19		20	20	4.5	Six weeks postoperative	
					90	150	177	87					Carcinoma of head of pancreas, obstructive jaundice	
10	1.7	+	8	25	93	113	92	20	7	9	8	1.5	After biliary fistula and drainage	
25	3.0	+							12	12	7	0.5	Carcinoma of papilla of Vater, obstructive jaundice	
					100				12	8		1.0	Twenty days postoperative, thirty two days postoperative	

Without Jaundice

Bile Index	Bile Pigments (Serum)		Coagulation Time		Fructose Tolerance, Blood Sugar, Mg, per Cent				Phenoltetrachlorophthalein				Dye in Urine, Mg	Comment
	Serum Bilirubin, Mg, per Cent	Van den Bergh Direct Reaction	Minutes	Seconds	Normal	1 Hour	2 Hours	Change	Dye in Serum, per Cent					
									15 Minutes	1 Hour	2 Hours			
5									12	8	2		Chronic cholecystitis, chronic cholangitis and hepatitis	
	1.1	0	5	40	123	163	160	40	12	7	3	0.3	Cholelithiasis, pancreatitis	
7	1.4	0			90	114	105	24	10	9	5	0.4	Chronic cholecystitis	
8	1.3	0			74	114	90	39	8	5	2	1.3		
9	0.8	±							10	6	2	0.6	Chronic cholecystitis with stones	
	1.5	0	9	10	85	174	122	89	8	5	4	0.3	Chronic cholecystitis, cholelithiasis	
	0.4	0	9						8	6	0	0.0	Former cholecystectomy, hepatitis	
			6	10					8	5	0	0.1	Former cholecystectomy, cholelithiasis	
8	1.4	0			107	115	96	8	8	4	0	0.8	Cholelithiasis, intermittent jaundice	
8	1.6	0			86	97	92	11	10	4	1	0.0	Chronic cholecystitis	
									8	4	2	0.3	Former cholecystectomy, chronic constipation	
6	1.1	0			71	87	80	16	8	4	0	0.0	Chronic cholecystitis, chronic appendicitis	
5	1.5	0			100	146	127	46	6	4	0	0.0	Former cholecystectomy, cholelithiasis	
5	0.9	0			98	107	92	9	10	3	0	0.4	Chronic cholecystitis, hepatitis, diabetes	
6	0.4	0	6	15	94	100	97	6	8	3	0	0.0	Chronic cholecystitis, hepatitis	
18	4.5	0	9	10	86	95	95	9	7	3	0	0.5	Chronic cholecystitis, hepatitis, pancreatitis	
									6	3	2	0.2	Chronic cholecystitis 48 hours after an acute attack	
8	0.6	0			93	105	85	12	5	2	0	0.0	Chronic cholecystitis	
	1.8	0			85	127	108	42		2	0	0.4	Chronic cholecystitis	
									7	1	0	0.4	Former cholecystectomy, postinfluenzal exhaustion	
5	2.0	0			86	83	88	2	6	1	0	0.2	Former cholecystectomy, hepatitis	
4	0.9	0			87	117	97	30	6	1	0	0.1	Former cholecystectomy, pancreatitis	
					160	133	110	-50	4	1	0	0.2	chronic arthritis	
													Former cholecystectomy, chronic nervous exhaustion	
	0.0	0			170				4	1	0		Chronic cholecystitis, hepatitis diabetes	
	0.8	0	9	50	97	120		23	3	1	0	0.9	Chronic cholecystitis, cholelithiasis	

CASE 5 (Case 40, Table 3) —*Obstructive jaundice, tumor of the head of the pancreas*—A man, aged 57, was first admitted to the Mayo Clinic Nov 2, 1923, because of pain in the right lumbar region of one week's duration. He had had occasional slight backache for several years and irregular epigastric pain one or two hours after eating for fifteen years. He had been losing weight, especially recently, and the right lumbar pain had become more severe. Physical signs and roentgenograms pointed to pleurisy at the right base. No other definite lesion was discovered, but after the patient's dismissal, the pain in the right upper quadrant and right lumbar region became quite severe. During these attacks the skin developed a subicteric tinge, though there was no definite jaundice. Urobilin and urobilinogen were present in the urine during the crises. Glycosuria and hyperglycemia were sometimes present.

On readmission, April 7, 1924, there was slight tenderness in the right upper quadrant, and an indefinite mass was palpated. Sugar, urobilin and urobilinogen were found in the urine. The serum bilirubin was 35 mg, the phenoltetrachlorophthalein reading was 4 per cent. The patient refused exploratory operation.

Jaundice and pruritus developed after the patient's dismissal from the clinic, and he returned May 18. He had lost 21 pounds (9.5 kg) in the preceding four weeks. Jaundice was present. The liver was palpable and the epigastric mass could still be felt. The urine contained bile and a trace of albumin. The serum bilirubin was 35.6 mg and there was marked retention of the phenoltetrachlorophthalein, the reading being 24 per cent. At operation an enlarged soft tumor in the head of the pancreas was found and the gallbladder was greatly distended with bile. There were no stones, and the obstruction was evidently due to the tumor in the head of the pancreas. A cholecystgastrostomy was performed, but the patient died on the third day of bronchopneumonia. Necropsy was not permitted.

The similarity between the changes in the laboratory findings in this patient and those seen in dogs following the experimental ligation of the common bile duct is worthy of especial note.

CASE 6 (Case 33, Table 3) —*Obstructive jaundice, carcinoma of the head of the pancreas*—A man, aged 62, came to the Mayo Clinic May 13, 1924, having suffered for five months from attacks of epigastric pain, which had begun with a sharp attack the previous January. He had lost 30 pounds (13.6 kg) since the onset, although there was no jaundice. The liver was palpable and a distended gallbladder could be felt below its margin. The urine contained albumin and pus cells. He had a sudden attack of sharp pain in the right upper quadrant during the examination. Three days later marked persistent jaundice developed. June 24, the serum bilirubin was 27.8 mg and there was marked retention of phenoltetrachlorophthalein, the reading being 44 per cent. The fructose tolerance test was questionable. At operation a diffuse carcinoma at the head of the pancreas was found to be obstructing the common bile duct. The gallbladder was distended with a great quantity of very thick black bile. The postoperative progress was not satisfactory. The blood urea increased to 91 mg on the fourteenth day, and the patient died July 17.

CHRONIC CHOLECYSTITIS WITHOUT JAUNDICE

Many patients with obstructive jaundice have had long standing cholecystic disease apart from the obstruction. The effect of this condition in relation to the changes observed in obstructive jaundice is best seen in the control series of cases of chronic cholecystitis shown in Table 4. In all of these, there was inflammatory disease in the right upper quadrant of the abdomen without icterus. Any differences between the findings in this series of cases and the three preceding may reasonably be ascribed to the effects of the bile retention.

CASE 7 (Case 67, Table 4) —*Chronic cholecystitis with cholelithiasis, hepatitis and pancreatitis (without obstruction)* —A woman, aged 46, was first observed Aug 16, 1923. She had had epigastric distress for three years. The early history was not characteristic, but eventually the pain became more severe and was referred along the right costal margin and to the shoulder. In June, 1924, the pain was colicky, and the patient became slightly jaundiced during the next few days. When she was seen, July 8, there was no jaundice and the liver was not palpable. The serum bilirubin was 0.8 mg and there was no retention of phenoltetrachlorophthalein. At operation chronic cholecystitis with multiple stones together with chronic appendicitis, hepatitis and pancreatitis was found. It was noteworthy that, with so extensive evidence of infection of the abdominal viscera including the liver, there should be no evidence of disturbance of the hepatic function revealed by the various tests.

CASE 8 (Case 58, Table 4) —*Chronic cholecystitis with cholelithiasis and pancreatitis (without obstruction)* —A man, aged 56, was admitted to the Mayo Clinic July 24, 1924. For twenty years he had had recurring attacks of colic in the upper part of the abdomen, with occasional attacks of slight jaundice and pale stools following the pain. In February, 1924, cholecystostomy was performed elsewhere and many stones were removed. Two months after the operation there was recurrence of the pain and the jaundice, associated with discharge of bile from the wound. The sinus persisted, and the pain and jaundice recurred periodically. At the time of admission there was no jaundice. The patient had lost a little weight. The liver was not palpable. The sinus was discharging bile and mucus. The serum bilirubin was 0.4 mg and there was no retention of phenoltetrachlorophthalein. At operation the gallbladder was found to contain several small stones and was removed.

DISCUSSION OF THE INDIVIDUAL TESTS

Fructose Tolerance —Tallerman⁸ has recently reviewed some of the difficulties incident to the use and interpretation of the results of the fructose tolerance test. He insists that the test shall not be considered positive unless the blood sugar reaches a value of 135 mg or more for each hundred cubic centimeters, with a rise of at least 30 mg in the sugar level. Our experience with normal persons has been similar to that of Tallerman, and we accept these criteria for a positive finding. At the same time, great care must be used in interpreting apparent deviations from this normal. In the presence of cholecystitis particularly, the possibility of an associated pancreatitis should be considered even in the absence of clinical diabetes.

In the twenty-eight tests performed on patients with uncomplicated obstructive jaundice, the fructose tolerance was definitely reduced in three instances. One of these patients had diabetes, in another evidence of pancreatitis was found at operation. In seven other patients, the blood sugar curve was suggestive, but did not fulfill the two criteria for a positive test.

Five patients with carcinoma of the head of the pancreas were studied, positive tests were obtained in three. The changes, however, may not have been entirely pancreatic in origin, for in one the fructose

⁸ Tallerman, K. H. The Levulose Test for Liver Efficiency and an Investigation of the Hepatic Condition in Pregnancy, *Quart J Med* **17** 37-52 (Oct) 1923.

tolerance returned to normal following cholecystostomy and relief of the obstruction

Of tests made on the group of patients with chronic cholecystitis, positive results were obtained in three. One of these patients had frank diabetes. A suggestive reaction was obtained in three other cases.

The fructose tolerance test apparently disclosed characteristic changes in experimental obstructive jaundice. Positive tests were not obtained clinically with any great degree of regularity in cases of obstructive jaundice. The additional difficulty of eliminating the possible effects of associated pancreatic disease have led us to question the clinical usefulness of this test.

Blood Sugar Level—No significant disturbance in the fasting blood sugar level was observed in the present series of cases, in only one instance was it below 85 mg, a control case in which there had been a previous cholecystectomy but in which there was no direct evidence of active hepatic disease. In only two cases was the fasting blood sugar above 150 mg, and clinical diabetes was present in one of these. Obstructive jaundice apparently has no marked effect on the mechanism regulating the blood sugar level.

Nitrogen Partition of the Blood—The changes in the nitrogen partition of the blood were in accord with the findings reported by Rowntree, Marshall and Chesney⁹. Retention phenomena were observed in one case in which renal insufficiency was a terminal event. The blood urea tended to be low in a certain percentage of these cases. In no case was it below 9 mg. The values lay within the lower range of normals, which perhaps was to be expected in view of the rather limited and often meat-free diet of many of the patients. The blood urea level on the average was apparently somewhat lower in the patients with icterus (from 22.5 to 28 mg) than it was in those with cholecystic disease alone (32.3 mg). This is in agreement with the observation that the blood urea level is reduced in dogs following the experimental ligation of the common bile duct. The changes observed in man, in our opinion, were not sufficiently well marked to be of clinical significance in the study of the individual patient, and the figures are not referred to in the case histories.

Bollman, Mann and Magath¹⁰ find that following hepatectomy in dogs urea formation ceases, and in consequence they assert that the production of urea is entirely dependent on the liver. A comparable degree of hepatic insufficiency was not observed clinically. In Case 2,

9 Rowntree, L. G., Marshall, E. K., Jr., and Chesney, A. M. Studies in Liver Function, *Tr. Am. Phys.* **29** 586-625, 1914.

10 Bollman, J. L., Mann, F. C., and Magath, T. B. Studies on the Physiology of the Liver, VIII, Effect of Total Removal of the Liver on the Formation of Urea, *Am. J. Physiol.* **69** 371-392 (July) 1924.

with a serum bilirubin of 16.2 mg, gross dye retention and an adequate urinary output, the blood urea rapidly rose from 30 to 146 mg with the development of marked toxemia. This suggests that impairment of one function of the liver, as that of bile excretion, is not necessarily paralleled by impairment in the other physiologic activities of that organ.

There was no noteworthy change in the other nonprotein nitrogenous constituents of the blood studied. The total nonprotein nitrogen, uric acid, creatinin and amino-nitrogen came within the limits of the normal.

Renal Function—Renal insufficiency has long been recognized as a postoperative complication of obstructive jaundice. Its importance has recently been emphasized by Walters and Parham¹¹ among others. Counseller¹² has been studying the morphologic changes in the kidney incident to bile retention. In general, however, in this series of cases we have not found evidence of serious renal damage apart from terminal or antemortem conditions. The normal phenolsulphonephthalein excretion serves further to emphasize the slight degree of impairment of renal function accompanying marked retention of bile. The majority of the patients with jaundice had varying degrees of albuminuria, usually slight, although casts frequently were present in the urine, but serious renal impairment was noted only as a terminal event.

Bile Pigments—Icterus is the most striking single clinical manifestation in patients with obstructive jaundice, and of itself affords specific evidence of functional disturbance in either the formation or excretion of bile. Van den Bergh,¹³ in particular, has pointed out what an important index the bilirubin content of the blood is to the degree of retention of pigment and to the clinical condition of the patient. Retention is evidenced in the blood before the development of the staining of the skin and sclerotics, characteristic of clinical icterus, and with the relief of obstruction the blood may return to normal before the disappearance of the color in the skin.

Patients with obstructive jaundice show an increase in the bilirubin content of the serum that is roughly proportional to the intensity of the staining of the conjunctivae. The highest value in this series of cases was 52.7 mg per cent, more than twenty-five times the maximal normal value. There was no essential difference between the cases in which the obstruction was due to stone or stricture and those in which it was due to carcinoma in the head of the pancreas. In such jaundiced patients bilirubin is the preponderant serum pigment and the "icterus index" shows variations proportional to the bilirubin content. As pointed out

11 Walters, W, and Parham, D. Renal and Hepatic Insufficiency in Obstructive Jaundice, Surg, Gynec & Obst 35 605-609 (Nov) 1922

12 Counseller, V S. Personal communication to the authors

13 Van den Bergh, A A H. Der Gallenfarbstoff im Blute, Leiden, van Doesburgh, 1918, 8

in the previous paper, the "icterus index" is of less value in patients with normal or slightly increased serum bilirubin because other serum pigments, especially those of vegetable origin, as from carrots, may result in a high index and so simulate bile retention. The serum bilirubin values were normal in those patients with chronic cholecystitis. It should be emphasized that the latter were studied during quiescent stages. We have found that such patients (Case 59, Table 4) often have a slight increase in the serum bilirubin during acute attacks, and this has been further emphasized by de Takáts¹⁴. This increase may not produce sufficient discoloration of the skin or conjunctivae to be clinically recognizable as icterus, but the study of the serum bilirubin by demonstrating the presence of this "latent icterus" or bilirubinemia often is of great diagnostic value. The determination is relatively simple and rapid and deserves a prominent place among the laboratory aids in the study and diagnosis of jaundice.

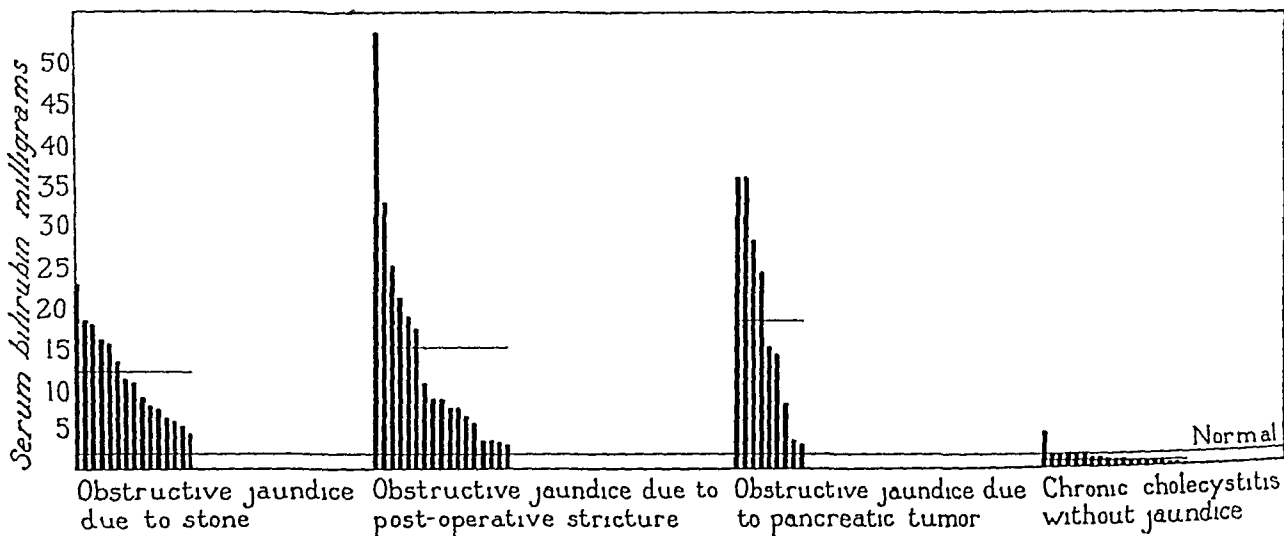


Fig 3—Variation in serum bilirubin in different types of obstructive jaundice. The maximal normal level is indicated, and in each group the average value is shown by the horizontal line.

Phenoltetrachlorophthalein—In the presence of an actual obstruction to the common duct, the study of the excretion of phenoltetrachlorophthalein in the stools or by duodenal drainage is manifestly impossible. The study of the rate of removal of this dye from the blood stream, as advocated by Rosenthal,¹⁵ greatly extends its clinical usefulness.

We have previously described the changes in the degree of dye retention following the experimental ligation of the common bile duct.

14 De Takats, Geza. Latent Jaundice as a Symptom of Biliary Colic, *Ann Surg* **81** 108-110 (Jan) 1925.

15 Rosenthal, S. M. An Improved Method for Using Phenoltetrachlorophthalein as a Liver Function Test, *J Pharm & Exper Therap* **19** 385-391 (June) 1922.

The changes observed clinically in cases of obstructive jaundice were similar. In all the cases of definite icterus there was marked dye retention. The maximal reading in the one hour specimen was 48 per cent, and in most cases it was 20 per cent or more, in marked contrast to the maximal normal reading of 3 per cent.

The cases of cholecystitis without jaundice for the most part did not show dye retention. In six there was slight retention and the dye reading in the one hour specimen varied between 6 and 8 per cent. This was perhaps to be explained on the basis of an associated hepatitis. In all the others the dye readings were normal.

The excretion of dye in the urine furnishes valuable confirmatory evidence regarding the degree of retention in the blood. Normal persons and those with cholecystitis may excrete traces of dye in the two hour

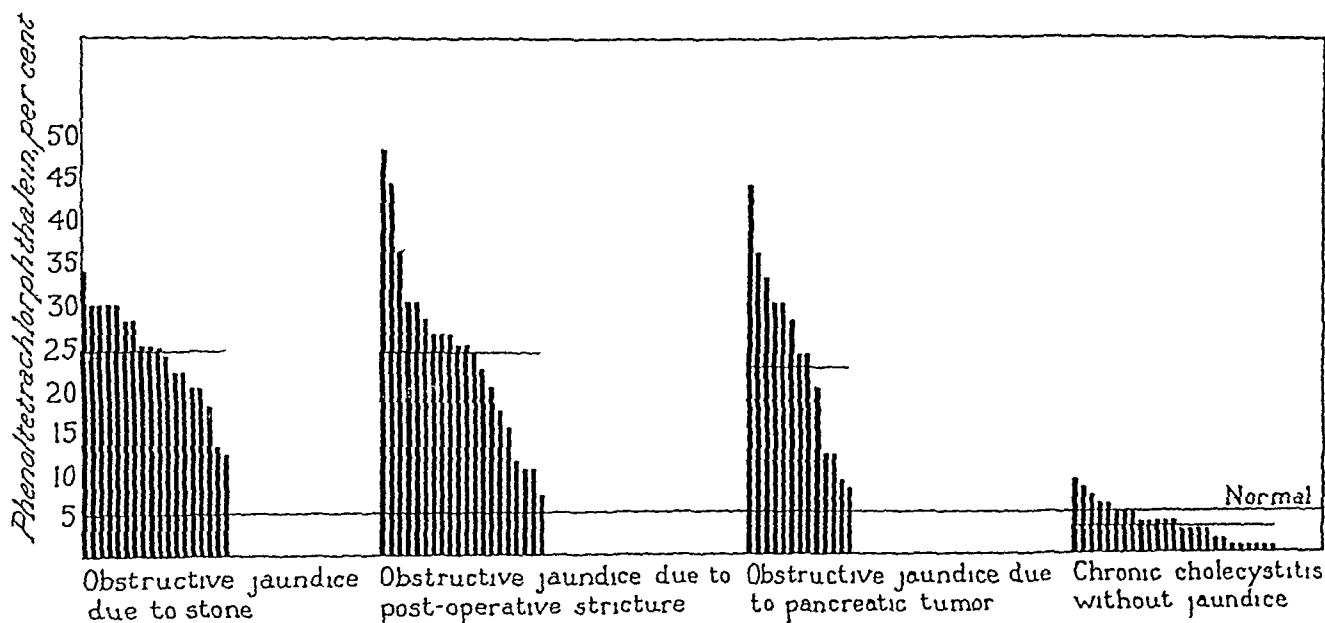


Fig 4—Variations in degree of phenoltetrachlorophthalein retention in the different types of obstructive jaundice. The maximal normal level is indicated, and in each group the average value is shown by the horizontal line.

urine specimen following the injection. In no instance, however, did the urine contain more than 1 mg of phenoltetrachlorophthalein without there likewise being marked dye retention in the blood stream.

COMMENT

Frerichs,¹⁶ in 1858, in his discussion of jaundice said

The bile which stagnates in the hepatic cells and bile ducts, in consequence of a mechanical impediment to its excretion, is carried into the blood by means of the veins and lymphatics. When the impediment has its seat in the ductus choledochus, the bile first shows itself after three days by a yellow color of

16 Frerichs, F. T. A Clinical Treatise on Diseases of the Liver. New Sydenham Society of London, 1850, 1: 93.

the conjunctiva Its absorption into the blood takes place much earlier, but in order that the jaundiced color of the skin should become visible, a certain amount of concentration is necessary The color makes its appearance in the renal secretion sooner than it does in the skin, and the serous effusions in the various cavities of the body become tinged with pigment earlier than the urine I have repeatedly found cholepyrrhin (bilirubin) in the serum of the blood and in effusions into the abdominal and thoracic cavities, where no traces of a jaundiced tint had been present either in the urine or in the skin

Apart from the tinging of its plasma, the blood undergoes no remarkable change from the absorption of bile, its proximate principles vary, as numerous analyses have shown, according to the constitution of the individual, and so forth, without presenting any constant abnormality

Recent advances in biochemical methods permit us greatly to amplify this statement of Frerichs A more detailed and exact study is now possible, but the conclusion is the same Hepatic derangement results from bile retention in the body but, in general, serious disturbance of carbohydrate and protein metabolism has not been demonstrated A diminished fructose tolerance is observed in a small proportion of cases and the blood urea level tends to be somewhat lower than usual, but the changes are not sufficiently striking to be of diagnostic import in any individual case This result may also be inferred from the small amount of liver tissue found by McMaster and Rous¹⁷ and others to be sufficient to support life

While changes in the intermediary metabolism of protein or carbohydrate are difficult of demonstration, disturbances in other physiologic activities of the liver may readily be shown The serum bilirubin and the phenoltetrachlorophthalein tests undergo characteristic changes The close parallelism between the degree of bile retention and of dye retention in cases of experimental obstruction of the common duct has already been pointed out Clinically there is much the same close relation between the intensity of the bilirubinemia and the degree of dye retention Case 5 is of particular interest because, when first studied, the serum bilirubin was only slightly increased while the phenoltetrachlorophthalein test was normal Following the development of obstruction, the serum bilirubin increased tenfold while there was marked dye retention Case 4 is likewise of interest because, when first seen, there was a decrease in the dye retention associated with the complete disappearance of previous icterus Later, following temporary obstruction and jaundice of only a week's duration, there was a definite increase in the degree of dye retention which became even more marked when complete obstruction developed Case 3 is even more interesting because it was possible to study the whole cycle of changes associated with the development of obstruction and its relief

17 McMaster, P. D., and Rous, P. The Biliary Obstruction Required to Produce Jaundice, *J. Exper. Med.* **33** 731 (June) 1921

The cases listed in the different tables show the stages in the return to normal following the operative relief of the obstruction. On the basis of our observations we may conclude that, immediately following the establishment of active biliary drainage, there is a rapid and progressive decrease in the serum bilirubin. Later the rate of removal becomes less rapid, and especially so if the prolonged jaundice has produced marked changes in the liver. As in Case 3, the serum bilirubin may be slightly increased over the normal for a considerable period.

The changes shown by the phenoltetrachlorophthalein test are similar to, but not identical with, those in the serum bilirubin. There is a rapid decrease in the degree of retention during the first few days. Thereafter there is a moderate degree of dye retention which is persistent, and the return to normal is very greatly delayed. During this period the degree of dye retention is greater than would be expected in comparison with the amount of bilirubinemia. The phenoltetrachlorophthalein findings may most readily be explained on the basis of the hepatitis described as occurring in cases of jaundice and observed at operation in many of the cases. The rapid changes immediately following the relief of the obstruction are perhaps evidences of a direct action of the retained bile on the liver. Experiments on the toxicity of bile, which will be reported later, apparently confirm this view. With the removal of the excess bile there is partial recovery, but the phenoltetrachlorophthalein test then indicates the persistent injury to the liver consequent on the jaundice.

The association of hepatitis with cholecystitis is well recognized. Graham¹⁸ and MacCarty and Jackson,¹⁹ among others, have described the lymphocytic infiltration and fibrosis present in the liver. These changes are most marked in the neighborhood of the gallbladder. It has been suggested that this hepatitis is responsible for the recurrence of symptoms in a small proportion of cases following cholecystectomy. The normal laboratory findings in the majority of the cases of cholecystitis, however, would indicate that this hepatitis does not produce any marked degree of functional disturbance.

We have stressed the close parallelism between the behavior of the serum bilirubin and the phenoltetrachlorophthalein in this series of cases. That the two readings do not have the same significance is shown by the behavior during the later portion of the recovery period. It is also demonstrated by the findings in cases of cirrhosis or carcinomatous involvement of the liver in which there may be marked dye retention without any bilirubinemia. These cases will be reported separately.

18 Graham, E. A. Hepatitis—A Constant Accompaniment of Cholecystitis, *Surg., Gynec. & Obst.* **26** 521-537 (May) 1918.

19 MacCarty, W. C., and Jackson, Arnold. The Relation of Hepatitis to Cholecystitis, *Minn. Med.* **4** 377-382 (June) 1921.

CONCLUSIONS

The greater number of tests of hepatic function studied fail to show significant, or sufficiently specific, changes to be of any great clinical value in the study of patients with obstructive jaundice. The fructose tolerance shows apparently characteristic changes following experimental obstruction to the biliary passages. Clinically, however, the results are too inconstant and the possibility of pancreatic effects too great for the test to be of value. The blood urea level, on the average, apparently is slightly lowered in cases of marked jaundice. This change again is too slight to be clinically significant in the study of any individual patient.

The most readily demonstrated changes in cases of obstructive jaundice are those in bile pigment metabolism. The icterus of itself is objective and conclusive evidence of disturbance in hepatic function. Changes in the serum bilirubin, however, precede the changes in the skin and conjunctiva. Accordingly, the determination of the serum bilirubin is the most sensitive and accurate index to changes in the degree of pigment retention, and so affords a quantitative guide to the progress of the jaundice. In addition, it permits the recognition of "latent icterus," which is of importance.

In cases of obstructive jaundice there is marked retention of phenol-tetrachlorophthalein in the blood stream that roughly corresponds to the degree of bile retention. This apparently indicates primarily the effect of the bile retention on the liver. After the complete disappearance of the jaundice and return of the serum bilirubin level to normal, there frequently is a persistent slight degree of dye retention that apparently indicates the degree of residual hepatic disturbance remaining in consequence of the jaundice.

These two tests give promise of definite clinical usefulness in the study, and particularly the quantitative study of the functional changes in patients with obstructive jaundice.

THE AURICLES IN CASES OF AURICULAR FIBRILLATION

CHANNING FROTHINGHAM, M D

BOSTON

Although a remarkable advance has been made in recent years in the knowledge of the irregularities of cardiac rhythm and of abnormal cardiac rates and very definite ideas have been established in regard to the mechanisms that underlie these abnormalities, very little has been added to the knowledge of the condition of the walls of the heart, especially of the auricles, which might be a factor in producing some of these disturbances in rate and rhythm. Many of these abnormalities in cardiac rate and rhythm are associated with disturbance in the normal action of the auricles, and still in most pathologic reports on the heart there is very little information given in regard to the condition of the auricular walls as compared with those of the ventricles. It is hoped that in the future pathologists will include in their routine description of the heart some information in regard to the degree of dilatation of the auricles, the thickness of their walls, and both the gross and the microscopic appearance of the muscle and other tissue in the walls of the auricles. This study was undertaken in order to find out if, in cases with auricular fibrillation, any lesions could be detected in the auricles which might be considered as a factor in the production of the fibrillation or as a result of it.

Before the nature of auricular fibrillation was as well understood as it is at present, a few workers had described lesions in the walls of the auricles in hearts with irregular rhythm. Among them Radasensky¹ in 1895 reported the appearance of fibrosis in the walls of the auricles in six cases in which the cardiac rhythm was irregular. Also, in 1899 Dehio² described fibrosis in the right auricle of hearts that had shown irregularity.

A few years later, as the polygraph began to show what was happening in the heart during auricular fibrillation, it was thought that the condition might be associated with some lesion in the neighborhood of or at the sino-auricular node, and the attention of several workers was directed to this region of the heart. As a result, Schonberg,³ Cohn and

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1 Radasensky *Ztschr f klin Med* **27** 381, 1895

2 Dehio *Deutsch Arch f klin Med* **62** 1, 1899

3 Schonberg *Frankfurt Ztschr f Path* **2** 153 1909

Lewis,⁴ Falconer and Dean,⁵ Draper⁶ and Hume⁷ all reported the presence of some lesions in this region. No one of these workers reported any characteristic acute lesion. In general an increase in connective or fatty tissues, chronic changes of the blood vessels or some infiltration with leukocytes were the lesions described. Price and McKenzie,⁸ on the other hand, did not consider that there was any more fibrosis in the region of the sino-auricular node in cases with auricular fibrillation than in those in which the cardiac rhythm was regular. In addition, Southerland and Coombs⁹ reported the presence of fat throughout the auricular musculature and an increase in endothelial cells in a case of auricular fibrillation associated with an acute infection. In this case, similar lesions also were present in the walls of the ventricles.

Apparently, interest in the attempt to put the cause of auricular fibrillation on an anatomic basis waned after these few studies, because one finds very little on this subject in the literature after 1914. Possibly this was due to the fact that subsequent studies on the nature of auricular fibrillation showed that it was not clear just what was going on and that very likely the trouble might not be with the sino-auricular node. Cohn,¹⁰ in Nelson's Loose Leaf System of Medicine, says that the pathologic lesions reported in the sinus region in cases of auricular fibrillation are not striking. He also mentions that the transient nature of auricular fibrillation in some cases suggests that there is no chronic organic lesion as the underlying cause.

Although at the present time it has not been definitely established in just what way fibrillation of the auricles begins and why it continues, a good review of the subject has been prepared by Garey¹¹ which brings out the various theories that have been advanced to account for the condition. In auricular fibrillation disturbances may occur in the pacemaker so that an abnormal number of impulses are sent out to the auricular muscle, or the control of the heart beat may be taken over by some other than the normal pacemaker. In addition, the conduction system for impulses in the auricular wall may be functioning in an abnormal manner. There has not been described any special tissue in the auricles for the transmission of impulses so it is presumed that the muscular tissue is the pathway along which travel the impulses to set off the muscular contractions. Finally, the muscle tissue itself in auricular

4 Cohn, A. E., and Lewis, T. *Heart* **4** 15, 1912-1913.

5 Falconer, H. W., and Dean, G. *Heart* **4** 87, 1912-1913, *ibid* **3** 247, 1911-1912.

6 Draper. *Heart* **3** 13, 1911-1912.

7 Hume, W. E. *Heart* **5** 25, 1913-1914.

8 Price, F. W., and McKenzie, I. *Heart* **3** 233, 1911-1912.

9 Southerland, G. A., and Coombs, C. F. *Heart* **5** 15, 1913-1914.

10 Cohn. *Nelson's Loose Leaf System of Medicine* **4** 333, 1920.

11 Garey. *Physiol. Rev.* **4** 215, 1924.

fibrillation may be in some way disturbed so that it does not respond to the stimuli in the usual manner. It is obvious therefore that with our present knowledge there is no one place in the auricles to which one might turn with the hope of finding a lesion to account for auricular fibrillation.

In this work, therefore, an attempt was made to study the auricles in the region of the sino-auricular node in order to see if there were any lesions about the pacemaker, and also to study the muscular tissue in other parts of the auricle in order to pick up, if possible, any lesions of the muscle that might be a factor in disturbing the passage of impulses or the response of the muscle to impulses. If any lesion should appear generally distributed throughout the muscle, it might, of course, be the result of auricular fibrillation and not a factor in the production of the condition. By a study of the relation of such a lesion to the duration of the auricular fibrillation, some idea might be formed as to whether a diffusely scattered lesion was the result or cause of the irregularity. Attention also was paid to the age of the subject as well as to the duration of the auricular fibrillation in order not to confuse lesions due to advancing years with changes in the auricular wall which might be the cause or result of this irregularity. In addition, the gross appearance of the auricles in regard to their size and the condition of their walls, as well as the size of the heart as a whole and any valvular defects or lesions in the ventricular walls, were studied in order to find if any peculiarities along these lines were associated with auricular fibrillation with sufficient constancy to be thought a factor in its production. As so little is described about the condition of the auricles in reports of necropsies, it was felt that careful control material representing similar ages to those of the patients with auricular fibrillation should be studied. In this work the material from control cases represented both acute and chronic disease.

The gross appearance of the cavities of the auricles, the thickness of the walls, the weight of the hearts and any valvular defects or lesions in the ventricular walls were noted in this compilation of cases. For microscopic study, tissue was taken from the region of the sino-auricular node, that is, between the entrance of the superior vena cava and the right auricular appendage and from the septum between the two auricles in the region of the foramen ovale, usually between the foramen and region of the sino-auricular node. In addition, a piece of the wall of the left auricle between the entrances of the pulmonary veins was studied and also a bit of tissue from the left auricular appendage. The tissues for microscopic study were fixed either in Zenker's fluid or 10 per cent formaldehyd solution and stained by eosin and methylene blue or hematoxylin and eosin, respectively, depending on the fixative.

In the group of cases that were studied there were eleven cases of auricular fibrillation associated with both acute and chronic diseases. In seven of these cases, the heart had been in constant auricular fibrillation for several days at least before death, and in one instance the auricular fibrillation had been present for several years. In three of the cases, the auricular fibrillation was a transient affair and in one instance apparently just a terminal phenomenon. The age of the subjects with auricular fibrillation varied from 21 years to over 70. The patient with fibrillation as a terminal event was just past 50 years of age, and the three patients with transient auricular fibrillation ranged from 40 to over 70 years of age. For comparison with these eleven cases with auricular fibrillation there were studied in a similar manner twenty-three others in which the heart action was regular. The ages of the control patients varied from 10 years to over 70 years, and they suffered from a variety of acute and chronic diseases.

Unfortunately, in many of the necropsy protocols no mention was made of the gross appearance of the auricles and, therefore, in these cases it had to be assumed that the auricles were approximately normal in appearance. It is appreciated that it would have been more satisfactory if an actual statement had been made that the auricles appeared normal, but there were sufficient notes on the abnormal appearance of the auricles in certain cases to justify the belief that if there had been anything striking in their appearance a note would have been made of it.

In the group of prolonged fibrillators, four of the seven cases presented evidence of dilatation of one or both auricles, but in the other three no mention was made of dilatation. In the four cases in which the auricular fibrillation was more transient in character, a dilatation of the right auricle in one instance was the only mention of that gross lesion. Of course, there may be several factors which can lead to auricular fibrillation, and dilatation of the auricles may be this factor in certain cases, but it seems quite evident from the foregoing figures that dilatation of one or both auricles is not essential to the production of this type of cardiac irregularity. That dilatation of the auricles may occur without auricular fibrillation is, of course, well known, and this was confirmed by the control cases in which seven out of twenty-three showed dilatation of one or both auricles.

In only three of the eleven cases of auricular fibrillation was any appreciable abnormality of the walls of the auricle recorded. In one of these three, the walls of both auricles were thought to be thinner than normal, in another the right auricular wall was thickened, and in the third the left auricular wall was described as thickened in one place and thinner than usual in another. Certainly with these meager findings which are also quite variable, one is not justified in concluding that

there is any gross disturbance in the auricular wall as yet described that is a factor in the production of auricular fibrillation

The weight of the heart as a whole was not essentially different in the cases with auricular fibrillation from the other cases. The weights of the hearts with the more prolonged fibrillation varied from 410 to 700 gm, with an average of 546 gm, which indicated a moderate enlargement. In the cases with more transient fibrillation the hearts varied from 240 to 520 gm, with an average of 357 gm. There is certainly nothing unusual enough about these weights to indicate that there is any relation between the size of the heart and this type of irregularity.

In this group with auricular fibrillation, there also were no lesions of the valves or myocardium of the ventricles which occurred with sufficient regularity to justify a belief that they are essential to the production of this type of irregularity. In the seven cases of more prolonged fibrillation, the valves and musculature of the ventricles were reported as normal in four. In one there was marked disturbance of the valves, in another there were chronic changes in the myocardium of the ventricles, and in the third an infarction of the wall of the ventricle. In the cases with more transient fibrillation, one of the three showed chronic changes in the musculature of the ventricular walls due to coronary disease, but in the other two there was no apparent chronic disease of the ventricular muscle or of the valves. In one of these two, there were yellowish streaks in the cardiac muscle, presumably due to the general sepsis that was present.

On the whole, with the data accumulated from these cases it is quite evident that there is no gross lesion in the heart that is essential to the production of auricular fibrillation. Although it is evident from clinical observation that stenosis of the mitral valve with subsequent dilatation of the auricles is often associated with fibrillation of the auricles, this irregularity was present in this series often enough without any valvular disease or dilatation of the auricles to rule out these conditions as essential.

In taking up the microscopic study of the tissues from the auricles, attention was first focused on the sino-auricular node region in order to see if any of the lesions described by the earlier workers could be found. In comparing the cases with fibrillation and those without in patients of the same age, it was quite apparent that there was no increase of connective tissue or definite scar formation in the tissues from the sino-auricular node region, and as far as could be determined the tissue that was thought to be the nodal tissue appeared the same in the fibrillators as in the controls. So far as the arteries were concerned there were some sclerotic changes in the blood vessels in certain cases, but these changes seemed to be related to the age of the patient and occurred

just as often in the cases without auricular fibrillation as with it. There was considerable edema of the tissues in this region in some instances, but it was not present in the majority of the cases with auricular fibrillation and also it was present in some of the controls. In the muscle tissue itself there was no lesion peculiar to this region, although a lesion did occur here as well as elsewhere in the muscle tissue of the auricles which will be described below. This lesion in the muscle tissue was present more often in the cases with auricular fibrillation than in the controls. In nine out of the eleven cases with fibrillation this degenerative lesion of the muscle was present. In fifteen control cases representing the same periods of age, this degenerative lesion in the muscle was only present in four instances. It is apparent therefore that this lesion in the muscle occurs more frequently in cases with auricular fibrillation than in cases without it. Although in a few instances there was some invasion of the connective tissue in this region by endothelial cells and leukocytes, it did not occur any more often in the cases with fibrillation than in the controls and in many instances was apparently associated with the other pathologic conditions present in the case, it therefore presumably was not of importance in regard to the cardiac irregularity.

These cases did not support the views advanced by most of the previous workers that there was a definite lesion in the region of the sino-auricular node in cases of auricular fibrillation. In the walls of the auricles away from this node there seemed to be no appreciable difference whether the tissue was taken from the left auricle or in the septum between the auricles. In general, the tissue taken from the left auricular appendage did not show quite so much as the other bits of the auricles in those instances in which some lesion was present in the muscle. Except for an occasional lesion that was obviously associated with the disease from which the patient suffered, irrespective of the cardiac condition, the abnormalities in the tissues were the same as those seen in the region of the sino-auricular node and occurred with the same relative frequency among the fibrillators and the controls. The interesting lesion in these cases was the one in the muscle fibers that was mentioned above.

The lesion that was generally distributed throughout the muscle of the auricles in certain cases varied considerably in intensity in the different cases. The slightest abnormality noted was an edema of the muscle fibers with vacuolization and some slight changes in the staining reaction of the fibers which suggested degeneration. When the process was more pronounced, the vacuolization increased, the striations in the fibers disappeared in part, and the changes in the staining reaction increased. The nuclei of the muscle fibers showed very little, if any, change and the disturbance in the fiber did not necessarily extend

throughout the whole of the individual fiber. In the instance in which the fibers showed the most marked disturbance, actual necrosis of part or all of the fiber took place and cellular reaction with phagocytes invading the muscle was in some instances present. It is rather striking how little if any cellular reaction was present in the milder forms of this degeneration of the muscle fibers. No evidence of appreciable reaction on the part of the supportive connective tissue framework was observed, nor was there any acute lesion made out in the blood vessels. In some instances there was marked interstitial edema as well as the lesion in the muscle. However, this interstitial edema occurred in other cases in which the lesion in the muscle was absent, so there may be no relation between the two. It seems probable that these various lesions are all different stages of the same process but, of course, it cannot be absolutely established that they are not the result of different causes. Just what the end-result of this degenerative lesion in the muscle will be could not be determined from these cases.

It is difficult to decide whether this degenerative lesion in the auricular muscle bears any relation to the production of auricular fibrillation or might result from it. The lesion does occur without auricular fibrillation but not with anywhere near as much frequency and not to such a marked degree. On the other hand, in two out of the eleven cases with fibrillation this lesion was not found. This lesion does not seem to represent a disturbance of old age, for in one case with auricular fibrillation of long standing in a patient over 70 years of age the lesion was not found, the lesion was present, however, at all ages among the fibrillators, and among the controls it was not confined to the older patients. That there is some relation between auricular fibrillation and this lesion is suggested by the frequency with which it was found in these cases in comparison to its occurrence in regularly beating hearts, but it may as well be the result of the auricular fibrillation as a factor in its production.

From the study of this small group of cases it was evident that there was no typical lesion in the auricles that could be looked on as the cause of auricular fibrillation. A slight degenerative lesion was found in the muscle fibers of the auricles much more frequently with auricular fibrillation than in regularly beating hearts of the same age. It is interesting to speculate whether this lesion was the result of the irregularity of rhythm or a factor in its production, by causing some defect in the conduction of impulses or in the ability of the muscle to respond to the impulses.

VENOUS PULSE PRESSURE

A CLINICAL NOTE¹

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In a recently published study of the centripetal venous pulse in man, White¹ discusses the unfruitful efforts to measure the pulse pressure in the veins. The residuum of pressure from the arterial side would appear insufficient to affect manometric readings through the intravenous cannula, in his judgment. Furthermore, the success of White's observations on the centripetal venous pulse depends on a proper equalization of pressure within the glass capsule with the diastolic venous pressure. The difficulties encountered in this direction warrant recounting the following case.

REPORT OF CASE

E G, a male student, aged 23, was admitted to the University Infirmary, March 28, 1924, in marked cardiac decompensation, manifested by persistent vomiting, dyspnea, cyanosis and palpitation. The underlying cardiac condition, which had existed for eleven years, consisted of double aortic and double mitral lesions. March 29, the symptoms of cardiac embarrassment became more pronounced and the venous pressure registered 18 cm of water. A venesection of 16 ounces (473.1 cc) was performed, with a fall of the venous pressure to 8 cm and marked general improvement. The arterial blood pressure was 160 mm systolic and 20 mm diastolic. For the first time a positive centripetal pulsation in the veins on the back of the hand became apparent.

The advantage gained by venesection was maintained. The venous pressure did not subsequently exceed 12 cm in spite of a complicating pericarditis which intervened April 1. The centripetal venous pulse continued until April 6. April 5, complete occlusion of the vein under observation was obtained at 12 cm. The vein collapsed at 8 cm, but with each systole there was a visible pulsation in the studied segment of the vein until a pressure of 12 cm was reached. Both end points were very distinct and were repeatedly confirmed.

This observation was interpreted to mean that during the cardiac systole a pressure of 12 cm of water existed in the peripheral veins and that on diastole this pressure fell to 8 cm, giving a venous pulse pressure of 4 cm. Obviously several factors, such as fever, heightened the venous pressure, and a dilated vascular bed in the presence of the excessive pulse pressure of aortic regurgitation combined to make such an observation possible in this subject, since repeated studies have failed to duplicate the foregoing findings. Indeed, the same subject four months later, having recovered his accustomed degree of cardiac compensation, did not show even the Quincke centripetal venous pulse.

* From the University of Wisconsin Medical School.

1 White, H L. Arch Int Med 35:124 (Jan) 1925.

Book Reviews

LA SIFILIDE IGNORATA E STRANA Second Edition By PROF C MARTELLI
Naples V Idelson, 1923

This valuable treatise belies its name. It not only deals with the rarer manifestations of syphilis, but also covers the entire subject.

The first half of the book consists of an orderly presentation of our present knowledge of the disease, the remainder is taken up with the more unusual types. The method is excellent from the standpoint of the student, as all but the barest outline of the clinical manifestations of the disease is included in the second portion, which is classified by anatomic systems. This has enabled the author to paint an unusually clear and vivid picture of the routine case in the first section, unencumbered by detail and variants.

The laboratory side of the problem is excellently presented, the sections on the spirochete, the Wassermann and other serologic and chemical tests being unusually clear and concise. In this connection, I wonder how many of us would dispose of the Lange colloidal gold reaction in a single sentence as "superfluous." The chapters on therapy cover not only the routine drugs used but also a thorough review of the chemotherapy of syphilis with a large variety of substances both old and new. Martelli is partial to the use of iodids, uses both inunctions and injections of mercury as a routine, and pushes the arsenicals rather heroically, not hesitating to give intramuscular injections in many cases. In the case of very young children, he leans toward conservatism. After his statements concerning the inability of the stomach to deal with adequate amounts of mercury in the adult, the prescription on Page 215, intended for the oral chemotherapy of children, seems rather fantastic.

In the second portion one might well take exception to much of the pathology described as syphilis. Diseases in patients with positive Wassermann reactions are not necessarily syphilitic, even though the patients do recover under antisiphilitic treatment. This is presumptive evidence that will not satisfy most scientists. There are 136 cases described in the volume as examples of disease. The author seems fully cognizant of the dangers of this method and speaks of "pansifilistas" whose enthusiasm induces them to ascribe all pathologic conditions to the spirochete. However, as a horrible example of this tendency, he quotes a case of frank syphilis, in which the patient developed inflamed joints, but declares that it was only rheumatism, because the patient recovered under salicylates¹. It is only fair to state, however, that most of the illustrative material is genuine, many cases being checked by operation, necropsy or other incontrovertible evidence.

The book is illustrated with many excellent photomicrographs and drawings, some in colors, but is marred by the inclusion of some wretched sketches of intravenous injections and a few poor photographs.

The indexing is excellent, and a very elaborate classified bibliography is included. The latter is noticeably lacking in references to the English and American literature.

PERNICIOUS ANEMIA AND APLASTIC ANEMIA By ARTHUR SHEARD, M.D. A
THESIS PRESENTED FOR THE DEGREE OF DOCTOR OF MEDICINE, UNIVERSITY OF
LEEDS New York, William Wood & Co., 1924

A detailed study is given of fifteen consecutive cases of pernicious anemia, one of hemolytic jaundice and one of aplastic anemia, and a review of ninety cases of pernicious anemia in the Leeds General Infirmary in the preceding ten years.

In the cases of pernicious anemia, the cord changes, achlorhydria and bone marrow changes are discussed in particular detail and the other features are

adequately considered. The author concludes that it is caused by an infectious agent in the gastro-intestinal tract, occurring only in subjects with achlorhydria. This is contrary to American ideas, as some cases of advanced pernicious anemia may retain a practically normal gastric acidity.

Aplastic anemia, the author believes, belongs to a pathologic syndrome, occurring either secondarily to toxic factors or primarily. It bears no relation to pernicious anemia. It may occur in all grades of severity and may show equal or disproportionate deficiency in the three blood elements, erythrocytes, leukocytes and platelets. Blood transfusions offer the only remedial hope.

PRECIS DE CLINIQUE SEMIOLOGIQUE. By GASTON LYON. Paris, Masson et Cie, 1924.

This excellent volume should make a good practitioner of its careful readers. While not pretentious, it takes up in a careful, systematic way the examination, diagnosis and treatment of patients. Beginning with a detailed chapter on history taking, physical examination and laboratory examinations, the author gives numerous valuable points with their significance clearly indicated. A detailed outline of system examinations follows, with interpretation of findings, special consideration being given to the diagnosis and the cause of symptoms. The digestive, respiratory, circulatory, urinary and nervous systems are dealt with at length. Prognosis receives careful consideration and the question of complications is well presented.

The detailed consideration of therapy includes chemical, physical, surgical, as well as colloidal, glandular and psychiatric methods. A chapter on the action of medicines follows, and the necessity of choosing the appropriate one from a number of possible drugs is emphasized.

Throughout is emphasized the need for careful and accurate study to determine not merely the diagnosis, such as pharyngitis, but also the underlying cause of the malady.

JAHRESBERICHT UBER DIE GESAMTE INNERE MEDIZIN UND IHRE GRENZGEBIETE. Berlin, Julius Springer, 1921.

This is an annual bibliography covering the entire field of internal medicine and biology. The major fields covered are general physiology and biology, pathology, diagnostic and symptomatic, therapy, pharmacology and toxicology, infection and parasitology, serology and immunity, metabolism, internal secretions, and a classification by systems of the digestive tract, liver and gallbladder, pancreas, spleen, urinogenital tract, blood, circulation, respiration, locomotion, and the neurologic system.

The volume is seven hundred and twenty pages. The references, predominantly German, include some American and French, but relatively few others.

BLOOD AND SYMPTOMATIC CHANGES FOLLOWING THE INTRAVENOUS ADMINISTRATION OF A VARIETY OF AGENTS AND SOLUTIONS*

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The object of this study was to observe the changes in composition and appearance of the blood occurring immediately after the intravenous injection of a variety of agents. These included some that cause anaphylactoid reactions in animals and some that are exploited for therapeutic purposes in human subjects. In this way, additional information regarding the effects of intravenous injections could be secured, and the hypothesis of hemoclasia or colloidoclasia, that is to say, disturbances in physical and chemical mechanisms of the blood and tissues, as the explanation of the changes and reactions could be tested. The analysis of blood was limited to the more important constituents and changes that might accompany such states as collapse, anoxemia, asphyxia, intravascular coagulation and thrombosis, which occur in varying degrees during the acute reactions from many agents injected intravenously. These have been described previously by several investigators. The literature of the subject has been reviewed in the publications of Hanzlik and Karsner¹ and recently brought up to date by one of us². It will therefore be omitted in this paper, except for a few of the more important and recent papers related to our work.

Numerous details recorded in these publications agree with the views that the anaphylactoid reactions rest on a physical-chemical basis, and that intravenous medication in general is dangerous. This holds even

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1 Hanzlik, P J, and Karsner, H T. *J Pharm & Exper Therap* **14** 379, 425 (Jan), 449, 463, 479 (Feb) 1920, *ibid* **23** 173, 237, 243 (April) 1924

2 Hanzlik, P J. *Basis of Allergic Phenomena*, *J A M A* **82** 2001 (June 21) 1924

for agents which appear to be relatively inert but which, nevertheless, act as foreign bodies in the blood stream and produce contact phenomena that determine the physical and chemical changes and mediate functional changes in the cells variously recognizable as symptoms and reactions. The results of the present study corroborate the views expressed by several investigators. In fact, they constitute a measure of proof of physical and chemical disturbances and confirm the limited reports on a few agents previously studied by others.

METHODS

In their studies on anaphylactoid phenomena, Hanzlik and Karsner¹ used guinea-pigs and some cats. In the present study, dogs were used throughout, because the extensive analyses that were made necessitated larger quantities of blood than can be obtained from smaller animals. Certain of the agents that were used, for example, histamin, are known to give different symptomatic responses in dogs than in guinea-pigs, but others, namely, arsphenamin, acacia, agar, copper sulphate and kaolin, were found by us to give practically identical responses in the two species. In work going on in this laboratory with pigeons, the symptomatic responses are similar to those of guinea-pigs, and the chemical and physical changes in the blood are similar to those of dogs. In the agglutination studies of Karsner and Hanzlik,¹ practically identical responses were obtained with cat, human and guinea-pig bloods. Hence, the general results in different species appear to agree fairly well and further justify the use of dogs in the present connection for obtaining an idea of the blood and tissue changes after the intravenous injection of agents causing anaphylactoid reactions.

Both morphinized and unmorphinized dogs with and without artificial respiration were used. When morphin was used, the dosage ranged from 0.25 to 0.5 c.c. of 4 per cent solution per kilogram subcutaneously. Light ether anesthesia was administered to all animals for the minor operative procedures that were carried out, namely, exposure and connection of the carotid artery for blood pressure record, insertion of a T-tracheal cannula and exposure of, and insertion of cannulas into, the saphenous vein and femoral artery. Then the ether anesthesia was discontinued during the remainder of the experiment. Kymographic records were made of blood pressure from a mercury manometer and of respiration from a tambour connected with a Woulff bottle, which, in turn, was joined to the tracheal cannula, the side arms of which was open so as to avoid asphyxia. Changes in temperature were recorded from a rectal thermometer. Careful records of symptoms and pulse and respiratory rates also were made.

All the agents and solutions were previously warmed and injected slowly into the saphenous vein from a buret. As a rule, the dosage was small and corresponded to that used for therapeutic purposes in human subjects. The doses of all agents used are recorded in the tables. In the majority of dogs more than one agent or solution was injected. This was done for economical reasons and also to observe changes under as many different conditions as possible. In many instances, the composition of the blood was changed by the first injection, so that the changes from subsequent injections of the same or different agents were additive. However, by reversing the order of injection of the agents, it was possible to secure first effects from all agents and also their effects under modified conditions of the preceding injections. In many instances, the changes produced were temporary and subsequent injections gave practically uncomplicated results. In any case, all conditions pertaining to such functions as respiration, blood pressure and temperature, and the composition of the blood were noted. In this way, we were able to secure economically numerous results under uncomplicated and complicated conditions, all of which served the purpose of the study. Certain complications, for example, circulatory collapse and

shock, which supervened on the first injection, served the purpose in that such conditions constitute indications for treatment by certain solutions (acacia and hypertonic sugar and salt solutions), according to their advocates. And it is in such conditions, rather than in health, that the blood, tissue and symptomatic changes are of practical interest.

Blood for the analysis was almost invariably drawn from the femoral artery through a long, bent glass cannula, the distal or free end of which was under from 10 to 15 cc of clean liquid petrolatum in a large test tube containing a little dry oxalate. In this way loss of carbon dioxide was reduced to the minimum. Between 15 and 17 cc was drawn with each bleeding, 15 cc being necessary in the analyses that were made. A control sample was always secured before an injection was made. The number of samples secured varied, but in general three or four were secured at fifteen minute intervals. In a few instances of fatal injections, only one or two samples were secured, and under these conditions blood was occasionally obtained from the heart. This is noted in the tables.

The following analyses were made as quickly as possible after the blood was drawn. The hydrogen ion concentration was estimated colorimetrically and electrometrically. In the colorimetric method, 1 cc. of blood was dialyzed under liquid petrolatum against 0.9 per cent sodium chloride solution, also under liquid petrolatum. At the end of from five to ten minutes, the dialysate was treated with phenolsulphonephthalein and matched at once against standards according to the method of Levy, Rowntree and Marriott.³ The colorimetric result was confirmed electrometrically as often as possible, and when the value appeared to be unusual or important. In the beginning of our work, we used 25 cc of blood for the electrometric measurements, but during the course of the work refinement in the apparatus and technique permitted us to use 0.5 cc for a large proportion of the measurements and eventually 'only 1 drop of blood was used. The electrometric technique has been previously described by De Eds and Hanzlik⁴ and need not be repeated here.

Suffice it to say that we took special precautions to control the accuracy of the results obtained by testing the apparatus frequently with standard phthalate (p_H 3.97) and with bloods of induced asphyxia. Hemoglobin estimations were made for the purpose of observing changes in blood volume, using 0.5 cc of blood diluted to 1 per cent solution according to Palmer's⁵ technique. Carbon dioxide content, and capacity (alkaline reserve) were estimated in 5 cc of plasma (centrifugalized and kept under liquid petrolatum) according to Van Slyke and Cullen's method⁶. Urea-nitrogen was estimated by the Van Slyke-Cullen modification⁷ of the urease-aeration method of Marshall, using 1 cc of blood. Ammonia was estimated according to Folin's⁸ aeration method in 1.5 cc of blood, many of the estimations having been made on samples that had been used previously for the electrometric estimations of p_H . For the ammonia, the sacrifice of blood was as small as possible, owing to the uncertain significance of small changes in its content. Lactic acid was estimated in 5 cc of blood colorimetrically with the aid of a Benedict colorimeter according to Harrop's⁹ method, using zinc lactate as the standard. Precipitation with 5 per cent copper sulphate solution was found to be most suitable for dog blood. The addition of 0.05 mg

3 Levy, R. L., Rowntree, L. G., and Marriott, W. M. A Simple Method for Determining Variations in the Hydrogen Ion Concentration of the Blood, *Arch Int Med* **16** 389 (Sept.) 1915.

4 De Eds, F., and Hanzlik, P. J. *J Biol Chem* **60** 355 (June) 1924.

5 Palmer, W. W. *J Biol Chem* **33** 119 (Jan.) 1918.

6 Van Slyke, D. D., and Cullen, G. E. *J Biol Chem* **30** 289 (June) 1917.

7 Van Slyke, D. D., and Cullen, G. E. A Permanent Preparation of Urease, and Its Use for Rapid and Accurate Determination of Urea, *J A M A* **62** 1558 (May 16) 1914.

8 Folin, O. A Laboratory Manual of Biologic Chemistry, D. Appleton & Co., 1916.

9 Harrop. *Proc Soc Exper Biol & Med* **17** 162, 1920.

of lactic acid to old serum was found to be the limit of sensitivity, 0.1 gm was readily detected and 0.5 mg in 5 cc, equivalent to 0.01 per cent concentration, gave a deep rose color. The normal lactic acid content of blood ranges from about 0.029 to 0.1 per cent, i. e., from 1 to 5 mg in 5 cc of blood.

The method, therefore, was sufficiently accurate for our purpose and decidedly advantageous over the time consuming extraction methods. Dextrose, phenol, acetic acid, alcohol and agar in blood gave negative tests with the reagents used in the method. Acacia gave a very slight pinkish yellow or brown and therefore not the characteristic color change. Hence, it appeared that these agents as constituents of the solutions that were injected did not contribute to the increases in lactic acid content when such were found. Total phosphate was estimated in 2.5 cc of blood according to the method of Bell and Doisy.¹⁰ Sugar was determined in 2 cc of blood by the picramic method of Benedict according to the technic of Myers and Bailey.¹¹

The sedimentation rate of corpuscles was obtained by comparing the column of corpuscles that separated from the plasma in a given time with the control samples of blood. This was done in both large and small test tubes. Frequently this phenomenon was very striking in comparison with the control tubes and merely required note of the fact. There were variations, of course, and under our conditions time was not available to evaluate these properly, the results are, therefore, recorded only with respect to an increase in rate of sedimentation or its absence. Almost invariably an increase in sedimentation rate was accompanied by agglutination, which could be recognized frequently in the supernatant plasma layer or in the suspension of a thin column of plasma in a small bore pipet. In doubtful cases microscopic examinations were made. Hemolysis was recognized from direct inspection of the plasma, and darkening of the blood by comparison with the control.

The important results and effects that were observed are recorded in the tables. A few tracing records of blood pressure and respiration are introduced to illustrate the changes in these functions. Physiologic analyses of these changes were not made as we were interested, for the time, in chemical changes. The effects of several of the agents in dogs are recorded for the first time. The extraordinary and unsuspected effects of some of the relatively inert agents on blood pressure and respiration, notably agar in low concentration, deserve further study. In several instances, the agents or solutions were used in high enough dosage or concentration to produce definite effects, so any effects short of definite ones produced by lower dosage merely emphasize the desirability of these chemical studies, which were made in order to reveal subtler changes that might be fundamental and produce moderate symptomatic changes, both subjective and objective and of importance clinically. The details of all results are discussed according to groups of agents. However, before proceeding to this, the changes from bleeding and asphyxia will be considered, since bleeding was a part of the routine in securing blood samples and is known to produce changes in blood composition. Asphyxia was used as a method of controlling changes in p_{H_2} , carbon dioxide, etc.

10 Bell, R. D., and Doisy, E. A. *J. Biol. Chem.* **44**: 55 (Oct.) 1920.

11 Myers, V. C., and Bailey, C. B. *J. Biol. Chem.* **24**: 147 (Feb.) 1916.

TABLE 1—Effects of Repeated Bleeding on Blood Composition and Symptoms *

Volume of Blood Drawn	Time, End of Min	Hemo- globin, per Cent	pH Colorimetric	CO ₂ Content		Urea Nitrogen, Mg in 100 C c	Lactic Acid, per Cent	Sugar, per Cent	Total Phosphate, Mg in 100 C c	Blood Pressure, Mg Mercury	Pulse Rate	Respiratory Rate	Rectal Temperature, C	General Symptoms	Sedimentation Rate	Agglutination and Other Changes
				Per Cent by Volume	Per Cent											
Experiment 31, Dog Weighing 6.5 Kg., One-Half Dose																
20		100	7.25	46.5 51.5		10.2	0.21	0.15	6.93	110		96	48			
15	30	98	7.2	45.3 49.4		10.0	0.22	0.14	5.04	106		58	30			
15	60	97	7.25	46.0 49.2		9.1	0.23	0.14	5.12	106		100	36			
15	90	102.6	7.3	45.1 48.2		10.0	0.23	0.13	4.82	108		100	36	None	Unchanged	None
15	120	99.0	7.3	44.2 46.2		10.5	0.21	0.15	5.25	104						
15	150	98.5	7.3	42.8 44.3		9.0	0.19	0.17	5.47	90		132	48			
Experiment 33, Dog Weighing 24.3 Kg., One-Half Dose																
15		100	7.1	50.0 51.2		11.2	0.14	0.07	3.5	90		96	60			
15	30	106.4	7.1	51.2 52.9		10.3	0.15	0.071	3.3	100						
15	60	111.1	7.15	51.2 52.9		10.8	0.17	0.081	3.4	100		100	64			
15	90	109.3	7.1	51.2 52.9		10.6	0.20	0.078	3.3	100		110	64	None	Unchanged	None
15	120	114.9	7.15	51.2 52.9		11.6	0.22	0.075	2.96	100						

* In the tables one-half dose equals one-half the full dose of morphin, the full dose being 0.5 cc. of 4 per cent solution per kilogram subcutaneously. In all cases in which no record is made it is understood that the dog received a full dose of morphin. The sign + means present or increase, —, absence or decrease, H hemolysis, D, darkening, art resp, artificial respiration, oc, occasional, Ag, agglutination, A, after, B, before, ? doubtful or questionable, and tr, trace.

BLEEDING AND ASPHYXIA

It can be stated at once that repeated bleeding as practiced in the experiments and to the end of two hours was not responsible for the results with the different agents that were injected. This is adequately illustrated by the results of Experiments 31 and 32 on two morphinized dogs presented in Table 1. The small variations in the changes pertaining to the blood constituents, including those for hemoglobin and carbon dioxide in Experiment 31, are considered to be within the range of experimental error. Although there was considerable accuracy in the analytic methods employed, under the conditions, the changes of 5 per cent, plus or minus, were generally disregarded, though they might be of significance in a particular case. Our conclusions are based on more pronounced changes. The changes and irregularities in blood pressure, pulse and respiratory rates during bleeding alone were too small to be of any significance. The rectal temperature of the one dog (Experiment 31) that was observed fell 2.4 degrees C. This is the tendency, however, in morphinized dogs.

The effects of asphyxia were observed in ten dogs, usually at the end of an experiment. Reproduction of the tabulation would serve no useful purpose because the changes observed were those that are well known, namely, increase in carbon dioxide and change of p_H toward acidity, these being the most important for our purpose. Illustrations of asphyxial changes are given in Table 2 under hexamethylenamin 4.7 per cent, and in Table 3 under 10 per cent sodium chloride (Dog 11), under 40 per cent hexamethylenamin (Dog 5), and under 85 per cent sucrose (Dog 10). In addition to these changes there were darkening of the blood, increase in lactic acid as a rule, and the usual changes in blood pressure, respiration and pulse rate. The asphyxia was induced by attaching a long rubber tube to the tracheal cannula. As far as lactic acid is concerned, Macleod¹² reported this not to be increased in blood when venous at the end of asphyxia, and that only about one-half the amount was usually present (0.019 per cent). On the other hand, he demonstrated an excess of blood lactic acid in anoxemia but not in surgical shock.

It is concluded that the repeated bleeding of small quantities of blood as practiced in our experiments produced no important changes in blood composition, therefore, this routine procedure was a negligible factor in the changes produced by the intravenous injection of the various agents that were studied. Asphyxia always produced definite changes in the appearance and reaction (p_H) and carbon dioxide content of the blood, and also some other changes. It served the purpose of a useful control of changes produced in the same direction by various agents in the absence of induced asphyxia.

12 Macleod, J. J. R. *Am J Physiol* 55:184 (March) 1921.

BALANCED AND ISOTONIC SOLUTIONS

The solutions used were 0.9 and 1 per cent sodium chlorid solution (five dogs), Tyrode's solution (one dog), and 4.7 per cent solution of hexamethylenamin (two dogs). The results are presented in Table 2.

Isotonic Sodium Chlorid and Tyrode's Solutions—With the sodium chlorid solutions, the principal effects consisted of variable blood dilution, roughly proportional to the volume injected, diminution in carbon dioxid, a slight and variable tendency toward acidity (lowering of p_H values), and very small increases in blood pressure and pulse rate in the majority of dogs. The fall in temperature was also slight, ranging from no change to 1.4 degrees C. When diminution in carbon dioxid occurred, this change could be accounted for by dilution of the blood. Tyrode's solution produced effects similar to those of sodium chlorid, except for the marked change in p_H at the end of thirty minutes. This low value of 7.1, however, was not confirmed electrometrically. A similar value was found in one out of five dogs receiving sodium chlorid solution.

Hexamethylenamin, 4.7 Per Cent Solution—This approximately isotonic solution of hexamethylenamin produced more marked changes in p_H toward acidity than the sodium chlorid solutions, the changes in blood dilution and blood pressure being similar to those after sodium chlorid. Carbon dioxid estimations were not made after hexamethylenamin. From this it would appear that hexamethylenamin, as a drug, had a more pronounced effect than sodium chlorid. Further on it will be seen that higher concentrations of hexamethylenamin produced even more marked effects.

The results with 0.9 and 1 per cent sodium chlorid and Tyrode's solutions indicate that these solutions, though isotonic and balanced, nevertheless may alter the blood and higher functions when they are injected intravenously. Therefore, they are not strictly physiologic, as would be expected from the considerable differences in their composition from that of blood, and as has been previously pointed out by physiologists. According to the recent report of Blumenstock and Luckhardt,¹³ 0.9 per cent sodium chlorid solution was the least physiologic solution of the three solutions used intravenously, namely, Ringer's, calcium-free Ringer's and 0.9 per cent sodium chlorid. Nine-tenths per cent sodium chlorid solution caused the most vigorous diuresis and the highest elimination of nitrogen. It often caused shivering. Fujimaki¹⁴ reports that the intravenous injection of from 20 to 25 c.c. of 0.9 per cent sodium chlorid solution daily into rabbits causes an increase in rectal temperature and of the carbon dioxid of the blood.

¹³ Blumenstock, J., and Luckhardt, A. B. *Am J Physiol* **67**:514 (Feb) 1924.

¹⁴ Fujimaki. *Arch f exper Path u Pharmakol* **103** 178, 1924.

17 (61)	32 8 c c	B	100	6 9	7 18	36 46	13 6	+	Ammonia +	100 140 6 min later	104	150	37 6	Asphyxial strug- gling	None	None
No mor- phin (1% sodium chlorid solution)		10 A	81 8	7 3	7 16	37 42	11 8			120	168		37 0	None		
		34 A	88 9	7 0	7 26	34 46	12 2	++	None	70	110	120	37 0			

Tyrode's Solution																
21 (61) 1/2 dose	16 4 c c	B	100	7 35	59 62	11 3	+++	Ammonia +++	40	72	24	33 6	None			
		15 A	88 5	7 4	55 59	11 9			60	96			None			
		15 A	85 1	7 1	52 55	11 5		+++	56	94			None			
	16 4 c c	55 A		7 2	52 58	12 5	+		60	94		31 8				

Hexamethylenamin, 4 7 per Cent Solution																
† (99)	10 1 c c	B	100	7 3	11 8	90										
		18 A	96 5	7 3	13 7	104										
		37 A	96 5	7 2	8 1	110										
		60 A	94 0	7 1	10 9	110										
		75 A	90 0	7 1	11 8	120										
† (101)	9 6 c c	B	100	7 25	7 23	130										
No mor- phin	Asphyxia	35 A	83 7	7 2	7 17	126										
		10 A	84 3	6 9	6 91	No record										
	Art resp	15 A	91 7	7 1	7 23	No record										
						Art resp										
						36 8										
						37 8										
						37 2										

* Phosphates were not estimated
† Data headed ammonia in this column in all tables means changes in ammonia in milligrams per hundred cubic centimeters of the blood, when the quantity is stated, all other data pertain to sugar
† The carbon dioxide data in this experiment mean total carbon dioxide content only

The increase in carbon dioxid is interpreted as alkali reserve, the alkali being called out in response to an increase in acidity. No observations of p_H were made, however. Such moderate disturbances in ionic equilibrium and state of the blood colloids as may be brought about by these solutions can conceivably account for alterations in functions. On the whole, therefore, when used in high dosage these solutions cannot be regarded as altogether harmless. However, the very small quantities used by us as solvents or vehicles for the various agents that were injected could not be responsible for the marked effects that were observed. They were the most practical vehicles for our use, whole blood, no doubt, being superior.

It is concluded that 0.9 and 1 per cent sodium chlorid solution and Tyrode's solutions when injected intravenously in doses of from 3.9 to 32.8 c c per kilogram cause dilution of the blood with a tendency to diminution of its carbon dioxid content, sometimes considerable change toward acidity (lowering of p_H), and the usual moderate increases in blood pressure and pulse rate. In small dosage, the effects are practically negligible. A solution of 4.7 per cent (isotonic) hexamethylenamin also produced dilution and a greater tendency to acidity of the blood.

HYPERTONIC SOLUTIONS

The solutions studied were sodium chlorid, 10 per cent, hexathylenamin, 40 per cent, sucrose, 85 per cent, dextrose, 50 per cent, and urea, 18 per cent. These solutions have all been injected intravenously in human subjects for various purposes. Certain ones have been exploited by Stejskal¹⁵ under the name of "osmotherapy." It is stated by Stejskal that the noninjuriousness of the following solutions and doses has been established for human subjects: from 10 to 20 c c of 10 per cent sodium chlorid solution, from 20 to 100 c c of 50 per cent dextrose solution, 20 c c of 50 per cent sucrose solution, 20 c c of from 10 to 20 per cent sodium sulphate solution, 20 c c of 20 per cent urea solution and from 10 to 20 c c of 10 per cent calcium chlorid solution. However, the enthusiastic claims of this author are not convincing. One hundred cubic centimeters of 10 per cent sodium chlorid solution has been injected in patients by Velden¹⁶ who states that it produces shortening of the coagulation time of blood with beneficial results in hemorrhage. Schenk¹⁷ states that he has confirmed this phenomenon with hypertonic salt solution and observed it with calcium chlorid. Blumel¹⁸ makes the same claim for oral administration of salt,

15 Stejskal. *Grundlagen der Osmotherapie*, Vienna, 1922, p. 215.

16 Velden. *Deutsche med. Wchnschr.* **35** 197, 1909, *Zentralbl. f. Herzkrankh. u. Gefasskrankh.* **11** 61, 1919.

17 Schenk. *Ztschr. f. d. ges. exper. Med.* **11** 166, 1920.

18 Blumel. *Med. Klin.* **2** 1175, 1910.

and Neuhof and Hirshfeld¹⁹ assert that intravenous administration of sodium citrate produces the same effect. The basis for the intravenous use of hypertonic sodium chlorid solution appears to be that tissue juice is drawn into blood along with tissue fluid in general. Intravenous injections of 200 cc of 15 per cent sodium chlorid solution in man have been practiced by Wynn,²⁰ of 15 and 30 per cent sodium chlorid solution by Cushing and Foley²¹ and by Sachs and Belcher.²² Barach, Mason and Jones²³ studied certain of blood changes caused by 15 per cent sodium chlorid solution in patients and rabbits. The venous oxygen in the three patients studied was markedly lowered, and the carbon dioxide content showed no constant or decided changes. Other changes were increase in blood or plasma volume. Chlorid content was constant in patients, but persistently increased in rabbits, fragility of corpuscles was not impaired, and one patient showed hemolysis. Various claims for and uses of 50 per cent dextrose solution (up to 1 gm per kilogram) will be found in papers by Burger and Hagemann,²⁴ Wells and Blankinship,²⁵ Litchfield,²⁶ Sansum²⁷ and Lo Monaco.²⁸ The great variety of effects, including blood changes, in animals that have been observed by many investigators will be found in the textbooks of pharmacology by Sollmann,²⁹ and by Meyer and Gottlieb,³⁰ who think that the nature of cell injury after hypertonic solutions is not understood and that chemical decompositions and cleavages occur. The results obtained by us on dogs are presented in Table 3.

Sodium Chlorid, 10 Per Cent Solution—The changes in blood produced by 10 per cent sodium chlorid solution were characterized by

19. Neuhof, H, and Hirshfeld, S. New York M J **133** 95 (Jan 15) 1921

20. Wynn, J. Intravenous Injections of Hypertonic Salt Solutions in Neurosyphilis, Arch Int Med **29** 72 (Jan) 1922

21. Cushing and Foley. Proc Soc Exper Biol & Med **17** 217, 1920

22. Sachs, E, and Belcher, G W. Use of Saturated Salt Solution Intravenously During Intracranial Operations, Preliminary Report, J A M A. **75**:667 (Sept 4) 1920

23. Barach, A L, Mason, W, and Jones, B P. Effect of Administration of Hypertonic Salt Solution on Blood Volume and Certain Related Blood Constituents, Arch Int. Med **30** 668 (Nov) 1922

24. Burger, M, and Hagemann, E. Deutsch med Wchnschr **47** 207 (Feb 24) 1921

25. Wells, C W, and Blankinship, R C. Intravenous Injections of Hypertonic Glucose Solution in Influenzal Pneumonia, J A M A **74** 75 (Jan 10) 1920

26. Litchfield, W F. Intravenous Glucose Injections in Shock, J A M A **69** 1410 (Oct 27) 1917

27. Sansum, W D. Glucose Injections, J A M A **68** 1885 (June 23) 1917.

28. Lo Monaco. Gior di med mil **66** 176, 1918, Gas med lomb, Milan, **76**.97, 1918, Rassegna di clin terap **17** 73, 1918, Presse méd **26** 617, 1918

29. Sollmann. Manual of Pharmacology, Ed 2, W. B Saunders Company, 1923

30. Meyer and Gottlieb. Experimentelle Pharmakologie, Ed 4, 1920, p 441.

TABLE 3—Effects of Sodium Chlorid (10 Per Cent), Hexamethylenamm (40 Per Cent), Sucrose (85 Per Cent), Dextrose (50 Per Cent), Urea (18 Per Cent), and Sodium Chlorid (0.1 Per Cent)

Dog No and Weight (kg)	Dose per Kilogram	Time of Blood Specimens in Minutes	Hemo- globin, per Cent	pH Colori- metric	CO ₂ Content			Sugar, per Cent	Blood Pressure, mm Mercury	Pulse Rate	Respira- tory Rate	Rectal Temper- ature °C	General Symp- toms	Sedimen- tation Rate	Aggluti- nation and Other Changes	
					pH Electro- metric	Per Cent by Volume	Urea Nitrogen, Mg in 100 C c									
Sodium Chlorid, 10 per Cent Solution																
3 (7.4) No mor- phin	3.4 c c	B	100	7.4		9.0			120							
		A	87.3 85.1	7.1 7.3		10.6 10.9		116 104								
	9.1 c c	B	100	7.2		11.5		136	78	210	36.8					
		9.1 c c preceded by dextrose, 100 c c	9 A	82.3	7.2		13.3		154	150	240					
			17 A	85.1	7.2	6.79	11.7		150	120	228	36.3				
36 A	85.1		7.2		12.7		140	84		36.6						
64 A	104.2		7.2		11.3		134	78	120	36.6						
11 (19)	9.1 c c	13 A	76.6	7.3		9.5		146	85				tremors,	+	H	
		23 A	75.2	7.1	6.77	12.7		140	130	40			struggling, twitching			
		49 A			7.2+	11.6		130	108				Increased reflex ex- citability	+	H	
		65 A	82.7	7.3		13.8		130			37.5	Tremors marked	+			
		Asphyxia	6 A	83.7	7.1	6.75	10.3		134			Convulsions				
15 (33.1)	7.6 c c preceded by 0.9 per cent sodium chlorid	B	91.3	7.20		10.8	+	0.1	110	60	14	35.6				
		10 A	86.6	7.15	7.11	11.1	+	140	112		34.2					
						52 56										
		30 A	85.8	7.0	7.03	11.4	++	140						Twitching, tremors		
		63 A	84.7	7.1	7.09	12.5		130	92	18	34.2	Twitching				
		98 A	89.7	7.1	7.17	11.2	++	120	60	18	34.0					

18 (82)	B 6 l e c preceded by 6 per cent acacia, 16 c c 30 A	68 69	7 04 7 04	54 9 62 3 53 1 56 8	12 5 12 1	+	Ammonia +	60 84	175 144	36 28	36 2 35 6	H Convul- sions, tremors
	68 A	69	7 0	51 2 54 9	13 3	++	++	80	153	30	35 2	H +
Hexamethylenamin, 40 per Cent Solution												
5 (72)	B 35 c c 139 gm 35 c c 139 gm Asphyxia	100 92 6 100 93 6 88 9 80 4	7 4 7 1 7 2 6 7 7 3 6 8	85 98 109 115 106 111				120 140 140 120 120 60	78 90 78 90	18 16 16+ deep	36 4 35 6 35 5	Struggling Twitching in legs Tremors, struggling
14 (66)	B 7 6 preceded by 50 c c sodium chlorid 10 per cent 3 03 gm 30 A 60 A Art resp 69 A 89 A	89 7 76 0 73 2 70 7 63 7 56 0	7 35 7 1 7 0 6 9 6 9+ 6 88	54 44 50 44 50 45 50 32 37 29 31	15 4 13 8 15 8 14 9 14 6 16 6			40 66 80 0 26 0	138 132 120	36 0 35 6 35 4	Tremors	Resuscita- tion with epinephrin Collapse, died

TABLE 3—Effects of Sodium Chlorid (10 Per Cent), Hexamethylenamin (40 Per Cent), Sucrose (85 Per Cent), Dextrose (50 Per Cent), Urea (18 Per Cent), and Sodium Chlorid (0.1 Per Cent) —(Continued)

Dog No and Weight (kg)	Dose per Kilogram	Time of Blood Specimens in Minutes	Hemo globin, per Cent	H ₂ O Colori- metric	H ₂ O 1 electro metric	CO ₂ Content			Lactic Acid, per Cent	Sugar, per Cent	Blood Pressure, Mm Mercury	Pulse Rate	Respira- tory Rate	Rectal Temper- ature C	General Sym- ptoms	Aggluti- nation and Other Rate Changes
						CO ₂ Capacity	Urea Nitrogen, Mg in 100 Cc	Per Cent by Volume								
15 (131)		B		7.2	7.23	43	10.3	17			120	60	16	33.8		
	6.6 c c	preceded by 200 c c 0.9 per cent sodium chlorid and 100 c c 50 per cent dextrose														
	2.66 gm	16 A		7.0	6.86	40	11.5				70 (6 minutes after injection)	120	36	33.4	Tremors, shivering	
		30 A		7.2	6.75	36	11.5				110				Continu- ous tremors	
		60 A		7.0	6.88	35	12.0	45			110	120	18	33.6		
													very deep, irregular			
16 (51)	9.8 c c	B	100	7.0	6.98	32	12.5	41			100	228		37.4		
No mor- phin	3.9 gm	10 A	77.5	6.9	6.89	37	14.6	10			40	156	21	36.4		
		24 A	77.8	6.8	6.8	30	14.6				70	141	21	36.4	Collapse	
	Art resp	89 A	81.5	6.8	6.65	33	13.8				40	72	Art resp	34.1	Strug- gling twitching	H
						24		33								
Sucrose, 85 per Cent Solution																
8 (86)	5.8 c c,	B	100	7.3			11.1				94	60	20	37.0		
No mor- phin	5.26 gm	10 A	78.4	7.3			11.1				120	78	Increased, deepened	36.5	Urination	
		29 A	102.6	7.3							114	70		36.6		
		108 A	112.4	7.1			8.4				110	120	24	37.2		
	5.8 c c,	10 A	88.5	7.3			9.7				120					
	5.26 gm	17 A	93.0	7.3			10.5				124	156		37.5	Twitching	
		30 A		7.3			9.7				110	144		37.0		

10 (178)	39 c.c. preceded by 50 c.c. hexamethylenamin, 10 per cent 3.22 gm.	B 5 A	99.0 67.0	7.3 7.3	7.22 7.22	0.8	120	132	12	26.0	Urination ...	D
		17 A	83.3	7.1	7.31	0.5	130		6	35.0	Twitching,
		20 A	95.2	7.3	7.22	0.5	130		Deep Decid	35.0	Twitching,
		47 A	94.8	7.2		8.0	160		10	35.0	Twitching,
		11 A	97.0	7.0	0.00	0.5	80		tube to trachea		Died	
		18 A	96.2	6.9	0.80	0.5						
Dextrose, 50 per Cent Solution												
11 (100)	5.26 c.c.; 2.63 gm.	B	100	7.0	7.0	11.7	134	110		37.8		...
		8 A	97.1	7.2	6.03	13.3	128	106		37.4		...
		10 A	99.5	7.21	7.02	15.0	131	108		37.4		...
		12 A	102.5	7.0	6.89	12.5	140	136		37.2		...
		60 A	109.5	7.21		13.0	131	96		38.8		...
		115 A	71.72	7.21		13.0	130	81				...
13 (04)	5.3 c.c.; 2.65 gm	171 A	70.02	7.2		11.5	120	78		35.0		...
		B	91.0	7.1	7.1		110	120	18			...
							126	120	10	35.0		...
							116	80	16	35.0		...
15 (131)	7.61 c.c. preceded by 93.8 c.c. sodium chloride, 10 per cent 1.87 gm	21 A	96.6	7.2			174	100	20	35.4		...
		61 A	84.0	7.1			120	60	18	34.0		...
		B	89.7	7.1	7.10	11.2					Twitching	...
							120	78		33.2	Twitching	...
		20 A	90.6	7.1	7.08	9.3	114	57		33.4	Twitching	...
		60 A		7.0	6.06	10.9	114	58	10	33.8		...
17 (131)		120 A		7.2	7.23	10.3	114	60		33.8		...
												...

* Figures represent carbon dioxide capacity of plasma

TABLE 3—Effects of Sodium Chlorid (10 Per Cent), Hexamethylenamin (40 Per Cent), Sucrose (85 Per Cent) Dextrose (50 Per Cent), Urea (18 Per Cent), and Sodium Chlorid (0.1 Per Cent)—(Continued)

Dog No and Weight (Kg.)	Dose per Kilogram	Time of Blood Specimens in Minutes	Hemo- globin, per Cent	pH Colori- metric	pH Electro- metric	CO ₂ Content		Urea Nitrogen, Mg in 100 Cc	Lactic Acid, per Cent	Sugar, per Cent †	Blood Pressure, Mm Mercury	Pulse Rate	Respira- tory Rate	Rectal Temper- ature C	General Sym- toms	Aggluti- nation and Other Rate Changes
						Per Cent by Volume	Per Cent by Volume									
Urea, 18 per Cent Solution																
16 (51)		B	51.5	6.8	6.66	$\frac{24}{33}$		13.8			40	72	Art resp	36.4		
	9.8 c.c. preceded by 50 c.c. hexamethylenamin, 10 per cent 1.76 gm	17 A	59.3	6.7	6.61	$\frac{24}{44}$		13.7			40	108	Spontaneous respiration Art resp	34.1	Twitching Struggling Quiet	H
		30 A	60.1	6.9	6.56	$\frac{27}{32}$		12.9			44	60	Art resp	32.8		H
		65 A	51.1	6.6	6.59	$\frac{27}{30}$					20	48				H + marked
19 (13.2)		B	96	7.0	7.09	$\frac{72}{70}$		11.1	++	++	120	70	20 36 180 20 minutes after injection)	36.2 35.6		H, D +
	7.58 c.c., 1.36 gm preceded by 16 c.c. per kilogram of 6 per cent sodium chlorid solution	33 A	90		6.62	$\frac{72}{74}$		10.3		+	150	114	Deep Irregular	35.4	Oc twitching marked	More H
		60 A	93	6.9	7.05	$\frac{70}{74}$		10.9	+		140	60	Irregular	35.2		Still more marked H
Sodium Chlorid, 0.1 per Cent Solution																
21 (61)		B	77.8	7.4		$\frac{52}{57}$		11.1	+	+	50	60 52		29.8		H
	16 c.c. preceded by 100 c.c. horse serum 31 A	71.7	7.2			$\frac{56}{51}$				+	60	47		29.2	Fascicular twitching of leg muscles	H marked
		60 A	67.5	7.2		$\frac{53}{60}$		12.2	++	++	50	48			No sedimen- tation, marked foaming	H marked
		74 A	62.6	7.3		$\frac{54}{60}$		11.5	++		40	40		28.2		H marked

considerable blood dilution, a tendency toward acidity (lowered p_H), and a reduction of carbon dioxide in the majority of four, and by hemolysis and an increased sedimentation rate in two dogs. There were demonstrable increases in lactic acid in the two dogs that were observed for this constituent, an increase in ammonia in one dog and practically no change in sugar in one dog. Urea was variable. Phosphate was not estimated. Acidosis in some dogs, also leukopenia, then marked lymphocytosis and a rise in temperature, are reported by Bacon, Novy and Eppler,³¹ who made intravenous injections of a hypertonic solution containing sodium chloride, 99 gm, calcium chloride, 2.86 gm, and potassium chloride, 3.3 gm, in 200 cc of water, injecting doses of 500 cc in from fifteen to twenty minutes.

Symptomatically, tremors, struggling, twitching and increased reflex excitability were observed in three of the four dogs and convulsions in one. These symptoms occurred generally when the changes in blood reaction, hemolysis and increased sedimentation rate were demonstrable. Blood pressure was elevated at once after injection in three dogs and somewhat lowered in one (Dog 3), pulse rate was variable, being accelerated in two and slowed in two dogs, while the respiratory rate tended to be slowed. The effects with 10 per cent sodium chloride solution were practically the same whether the solution was given alone or preceded by other solutions (as in Dog 15, by 0.9 per cent sodium chloride solution and in Dog 18 by 6 per cent acacia).

The symptomatic effects observed by us agree with the well known effects of hypertonic sodium chloride solution. They are generally ascribed to dehydration, changes in osmotic pressure and in ionic balance in the tissues. These factors undoubtedly play a part, but isotonic (0.9 per cent) sodium chloride solution also produces physiologic changes, as indicated in the preceding section. The osmotic factor is not so prominent in isotonic saline solution, though, of course, such a solution does not possess other ions in balance necessary for the tissues. The changes in p_H observed by us indicate a disturbed balance of carbonate and phosphate ions in the blood in part, and in part alterations in diffusion phenomena and surfaces. The increased rate of sedimentation would be expected from dilution of the blood, but when it is accompanied by hemolysis, as was the case in two dogs, this phenomenon indicates some surface change in the cells. The decrease in carbon dioxide can be accounted for by dilution of the blood, but the dilution does not adequately account for the change in p_H toward acidity. The acidity may be accounted for, in part at least, by the lactic acid, which was increased in two dogs. However, the blood of the other two dogs was not tested.

31 Bacon, D. K., Novy, F. O., and Eppler, H. H. Factors in Leukocytosis, *Arch. Int. Med.* 30: 229 (Aug.) 1922.

for lactic acid Total phosphate was not estimated, and therefore the evidence for an increase of fixed acids is incomplete Urea remained unchanged or was variable in accordance with the well known tendency of this metabolite to maintain even distribution between the blood and tissues

As far as the other blood constituents that were studied are concerned, there is no doubt that intravenous injection of 10 per cent sodium chlorid solution causes considerable disturbance, and it appears that the symptomatic changes are dependent on these physical and chemical changes As far as we know, the changes in p_H and carbon dioxid have not been previously described It will be seen later on that they can be brought in line with similar changes produced by hypertonic solutions of other nontoxic and toxic agents and with isotonic solutions and suspensions of relatively inert agents and colloids All this indicates that the mechanism of action of these nontoxic, inert and colloidal agents is concerned with disturbances in physical and chemical mechanisms of the blood and tissues, in the same sense as those involved in the action of hypertonic sodium chlorid solution

Hexamethylenamin, 40 Per Cent Solution—Nineteen injections of 40 per cent hexamethylenamin solution were made into twelve dogs, but in order to save space the results in only four dogs are presented in Table 3 The results of all the injections were practically the same and are well typified by those presented Qualitatively the effects produced were essentially the same as those of 10 per cent sodium chlorid solution and the other hypertonic solutions that were injected, but quantitatively they were more pronounced There was a greater and more lasting dilution of the blood, a greater and perhaps more lasting tendency toward acidity, more marked decrease in carbon dioxid and alkali reserve The urea was variable Lactic acid, sugar and phosphate were not estimated As for symptoms, these were more pronounced than with the other hypertonic solutions The blood pressure and pulse rate were increased in two and decreased in two dogs Collapse supervened in two (Dogs 14 and 16), necessitating resuscitation by artificial respiration The respiration tended to be slowed, deepened and irregular in two (Dogs 5 and 15), and in Dogs 14 and 16 respiration stopped at the end of sixty and eighty-nine minutes, respectively, and they required artificial respiration Struggling and tremors and twitching in the legs and other parts of the body were present in all the dogs Death occurred at the end of eighty-nine minutes after injection of the hexamethylenamin in Dog 14, which had previously received 10 per cent sodium chlorid solution This dog had a shock level of blood pressure before the injection of hexamethylenamin which raised it 100 per cent temporarily However, Dog 16, which had received no morphin or solutions prior to hexamethylenamin and had a high level of blood pressure at the start

responded to the hexamethylenamin with a shock level of pressure and required artificial respiration. From this it appears that the hypertonic hexamethylenamin solution was responsible for the marked collapse in these two animals. The tremors, struggling and twitching were undoubtedly more pronounced with the hexamethylenamin than with the other hypertonic solutions, these symptoms occurring usually when the blood p_H values were at their lowest. Changes in agglutination, hemolysis, etc., were not consistently observed, though hemolysis was noted once at the end of the experiment in Dog 16.

All changes occurring after intravenous injection of 40 per cent hexamethylenamin indicate that this solution is injurious. It is this concentration of the drug that has been exploited by the enthusiasts of intravenous therapy for therapeutic purposes in human subjects with complete disregard of the fact that all its ordinary urinary antiseptic effects can be secured from oral administration, owing to its rapid absorption, and urinary excretion. The disturbances produced on the intravenous injection of the drug are not due to formaldehyd liberation, despite the occurrence of a slight acidity following its injection, for the following reasons: (1) the acidity would not be high enough to cause an adequate liberation of formaldehyd, (2) the slight and momentary liberation of free formaldehyd would be rendered inert by combination with proteins and by oxidation to formic acid, and (3) actual tests made by one of us (F. D.) under optimal conditions of blood acidity during asphyxia did not reveal even a trace of free formaldehyd in the blood.³² The cause of these changes, therefore, is the hexamethylenamin itself, and this despite the fact that the drug is nontoxic in the ordinary sense. Fundamentally, the cause of the changes from hexamethylenamin given intravenously appears to be the same as that of intravenous injections in general, irrespective of the physical and chemical nature of the agent injected.

Sucrose, 85 Per Cent Solution—Simple syrup (U. S. P.) was prepared from chemically pure sucrose and injected intravenously in two dogs. The results are not as complete (Table 3) as with dextrose, and in view of the less pronounced effects the experiments were not extended. Data for carbon dioxide, sugar, lactic acid and phosphates are scattering and incomplete not only with sucrose, but also with other agents in Table 3 because these were our earliest experiments.³³ Dog 8 (unmorphinized) received two successive doses of sucrose alone. Ten minutes after each injection, there was considerable blood dilution from

32 De Eds, F. Fate of Hexamethylenamin in Body and Its Bearing on Systemic Antisepsis, *Arch. Int. Med.* **34** 511 (Oct.) 1924.

33 At the time the full significance of the changes we were finding was not appreciated, nor was our application of the several methods, which had to be applied simultaneously, yet satisfactory enough to give reliable results.

which there was a prompt recovery. The p_H remained practically unchanged, except at the end of 168 minutes after the first injection when it was found to be 7.1, but this was not confirmed electrometrically and may have been accidental. The urea showed unimportant variations. On the other hand, Dog 10 (unmorphinized), which had recovered from an injection of 40 per cent hexamethylenamin, responded more markedly to about three-fifths the dose of sucrose used in Dog 8. The blood dilution was more marked and lasting and the p_H values fell steadily, indicating a tendency toward acidity. The animal was killed by asphyxia. As for symptoms, these were about the same in both dogs. Both dogs urinated copiously and showed twitchings, struggling being present in Dog 10, which had received hexamethylenamin previously. The blood pressure and pulse rate were increased. The respiration was deepened in both dogs, slowed in one and somewhat accelerated in the other. The rectal temperature fell slightly.

Dextrose, 50 Per Cent Solution—Of the three dogs that were injected, Dog 11 received dextrose alone, Dog 13, following injections of 0.9 per cent sodium chlorid solution, and Dog 15, after 10 per cent sodium chlorid solution. On the whole, the results, though incomplete, were about the same as those after sucrose alone. Temporary dilution of the blood occurred in all the dogs, this being more marked in the dogs receiving previous injections of the sodium chlorid solutions. The p_H values tended to increase (tendency toward alkalinity) or remained practically unchanged. The dilution of the blood could explain the lowered carbon dioxide content. According to Fujimaki,³⁴ one-half the concentration (25 per cent) of dextrose used by us increased the carbon dioxide of the blood in rabbits. Intravenous injection of 20 per cent dextrose was found not to increase the reserve alkali (carbon dioxide) capacity of the blood by Tatum.³⁵ The urea in our dogs showed minor variations as usual. Lactic acid, sugar and phosphate were not estimated. In 1893, Harley³⁶ reported a marked increase of lactic acid soon after injection of 10 gm per kilogram of dextrose (50 per cent solution) intravenously in dogs, at the same time, the glycogen of the liver and muscle were reduced and acetone appeared in the breath. According to Harley, these changes occurred when the blood sugar reached 1 per cent of the body weight. In a second report in 1894, Harley³⁷ described an increase in lactic acid of the blood during the first hour and reduced tension of oxygen during the first two hours after intravenous injection of dextrose. According to him, the lactic acid would bind the sodium of the blood and result in reduction of carbon dioxide,

34 Fujimaki Arch f exper Path u Pharmacol **102** 236, 1924

35 Tatum, A. L. J Biol Chem **41** 59 (Jan) 1920

36 Harley DuBois, Arch f Physiol Supplement, p 46, 1893

37 Harley DuBois, Arch f Physiol, p 451, 1894

which would be eliminated by the increased respiration. Besides an increase in respiration, Harley observed fibrillary twitching, vomiting, motor depression and contracted pupils. On the following day the behavior was normal. In 1917 Macleod and Fulk³⁸ reported an increase in hydrogen ion concentration of blood after injections of dextrose. Hyperglycemia and acidosis frequently go together. The symptoms observed by us were mild, showing only small and temporary increases in blood pressure and pulse rate. Respiration was slightly slowed in Dogs 13 and 15, twitching and tremors were observed only in Dog 15 which had received 10 per cent sodium chloride solution previously. In this dog there was also an increase in sedimentation rate of the corpuscles.

As far as our results go, it would seem that 85 per cent sucrose solution and 50 per cent dextrose solution do not alter the blood very much, except for some dilution by both sugars and lowering of carbon dioxide by dextrose, due very likely to the dilution. After sucrose the reaction (p_H) remained practically unaltered, and after dextrose the tendency was to maintain or increase the alkalinity, though not above normal, this may be regarded as a beneficial change. Presumably, the dextrose was more readily burned to carbon dioxide than the sucrose, and this gas increased the alkali reserve though the carbon dioxide capacity did not show such an increase. There are other possibilities but without further analysis of the causes for these changes, speculation is useless. It is interesting to note, however, that as the blood dilution was recovered from (the blood concentration increased) the carbon dioxide nevertheless kept on decreasing. Perhaps twitching was more marked after sucrose, and the remaining functions that were observed were improved somewhat or not seriously impaired. It is noteworthy that the dogs that had received hypertonic solutions prior to the sugar solutions exhibited more pronounced tremors, struggling or twitching.

Urea, 18 Per Cent Solution—This solution was injected into two dogs of which one (Dog 16) had previously received 40 per cent hexamethylenamin solution and one (Dog 19) 6 per cent acacia solution. Blood dilution was marked in the dog receiving the hexamethylenamin previous to the urea solution, and only slight in the dog that had previously received acacia. The p_H values showed a tendency to decrease, though only slightly in the dog that had received acacia. The carbon dioxide and alkali reserve were increased in Dog 16 receiving hexamethylenamin and slightly increased in Dog 19, receiving acacia, before the urea. It appears, therefore, that the increase in carbon dioxide in Dog 16 may be responsible for the further increase in acidity (further decrease of p_H) while the decrease in the carbon dioxide in Dog 19

38 Macleod, J. J. R., and Fulk, M. E. *Am J Physiol* **42** 193 (Jan.) 1917

may be accounted for by blood dilution, which also may account for the decreases in lactic acid and sugar. The urea content of the blood was not increased, the values remained within the normal range despite injections of large doses of the metabolite. This result agrees with the well known behavior of injected urea. The condition of shock present in Dog 16 was probably responsible for the original low p_H values of the blood in this animal. However, the injection of the hypertonic urea solution did not improve the blood pressure, although the pulse rate and respiration were temporarily improved. In fact, the collapse soon became worse, asphyxia supervened, and the carbon dioxide of the blood increased. Other symptoms in this dog were a marked fall of temperature, twitching and struggling. The sedimentation rate of corpuscles increased and hemolysis was present. In Dog 19 the symptoms were characterized by temporary increases in blood pressure and pulse rate and by a rapid, then irregular and deepened respiration. Occasional twitching was present and the rectal temperature fell 1 degree at the end of one hour. The sedimentation rate and hemolysis of corpuscles were decidedly more marked in the blood samples after the injection of urea than in the preceding samples after acacia alone. The darkening produced by acacia was absent in the blood specimens after injection of urea. Therefore, all effects that have been described were due to the urea, the previous conditions in the dogs produced by the hexamethylenamin and the acacia being aggravated if anything by the urea. However, the effects of the 18 per cent urea solution are not peculiar, but are in the same general direction as those of other hypertonic solutions, except for the more pronounced hemolysis, as may be expected.

The conclusion is justified that hypertonic solutions of sodium chlorid (10 per cent solution), hexamethylenamin (40 per cent solution) and urea (18 per cent solution), produce definite changes in blood composition, such changes being less evident, partial or even absent after sucrose (85 per cent solution) and dextrose (50 per cent solution). With the sugars the chemical evidence is not as complete as would be desired. The most marked effects and symptoms were produced by hypertonic hexamethylenamin solution, and the least by the hypertonic solutions of the sugars.

HYPOTONIC SODIUM CHLORID

The effects of 0.1 per cent sodium chlorid solution were observed in a dog (Dog 21, Table 3) that had received 100 c.c. of normal horse serum one hour before. Considerable disturbance occurred. The blood showed marked dilution, a fall of p_H , increases in carbon dioxide, lactic acid and dextrose and a practically unaltered urea. The blood pressure rose moderately and temporarily and the pulse rate was slowed, the rectal temperature fell and struggling and fascicular twitching of the

muscles were definite. Hemolysis was marked, being considerably increased over some hemolysis produced by the horse serum. When shaken the blood foamed markedly, a phenomenon not observed in other bloods of this study. The effects corresponded somewhat to those of asphyxia, although the blood was not particularly dark. The increases in carbon dioxide and lactic acid were very likely responsible for the lowering of the p_H values (acidity), in spite of blood dilution. The fascicular twitching and struggling were present when the p_H values were low. There is no doubt, therefore, that the intravenous injection of hypotonic (0.1 per cent) sodium chloride solution produced definite blood and symptomatic changes. This was to be expected and is a fact, in part, well known.

COLLOIDAL SOLUTIONS

All the solutions described thus far, except hexamethylenamine, have been studied previously by others with respect to many of the changes observed by us. The results obtained, therefore, serve as a check on the methods employed and further sustain the correctness of all the changes with the solutions to be described in this and the succeeding sections. The changes produced by the majority of these have not been previously reported, and certainly not with the objective constantly before us in this study. Several of the agents to be described produced anaphylactoid phenomena in guinea-pigs, and certain of them are exploited or recommended for intravenous use in human subjects. Among those relatively inert in the ordinary sense are the emulsoid colloids, whose effects are described in this section. Considerable importance is attached to the results with the colloidal agents, because despite the assumed inertness they produce definite blood changes and symptomatic effects ranging from the just demonstrable effect to death.

Agar Sol Gel, 0.1 Per Cent—One-tenth per cent of agar solution was boiled in 0.9 per cent sodium chloride solution without loss of volume until the strands disappeared. Then the mixture was filtered to remove debris. The filtrate was opaque, the agar being in the form of a gel in part, and in part in the sol state. There were no coarse aggregates. It was kept and injected warm at 38 degrees C. Such an agar mixture when injected intravenously into guinea-pigs produces violent reactions and death. The three dogs injected by us responded with marked symptoms, two of them died. The dosage of the agar in all three animals was very small, i. e., from 0.01 to 0.0125 gm per kilogram of body weight. Apparently small dosage is not a serious drawback in the production of agar reactions. On the contrary, it appears to favor their elicitation provided the agar is finely dispersed. In Boïdet's experiment with "toxified agar," for instance, the quantity of agar in the clear supernatant fluid after centrifugation is too small for

TABLE 4—Effects of Agar Sol Gel (0.1 Per Cent), Acacia (6 Per Cent and 25 Per Cent), Dextrose (18 Per Cent), Gelatin (5 Per Cent) and Horse Serum

Dose No and Weight, kilogram	Time of Hemo Blood globin, per Specimen, Min	p _H Color, metric	p _H Electro metric	CO ₂ Content		Lactic Acid, per Cent	Sugar, per Cent	Total Phos, Mg in 100 Cc	Blood Pressure, mm Mercury	Pulse Rate	Respi ratory Rate	Rectal Temperature, °C	General Symptoms	Sedimen tation Rate	Aggluti nation and Other Changes
				CO ₂ Capacity Per Cent by Volume	Urea Nitrogen, Mg in 100 Cc										
Agar Sol Gel, 0.1 per Cent															
24 (5.4)	B	106.4	7.2	35 36	10.9	0.1	0.25	Decreased ↓	40	110	Art resp throughout	36.8	Resuscitat ed from collapse	—	
10 c c, preceded by shock and collapse 0.01 gm				16 A	104.2	7.1			30	108	Some spontaneous respiration	35.4		+	
				36 A	103.6	7.1			24	120		36.2		+	
				91 A	81.0	6.8		Increased	10				Died		
25 (40)	B	100	7.15 (7.15, plasma)	7.11 (7.15, plasma)	9.3	0.09	0.13		80, left carotid 10, right carotid 10	104 101	15 21	34.6 immediate effect			
12.5 c c, 0.0125 gm				21 A 26 A	85.5 77.5	6.95 7.1	0.06 0.06		16 10	90 58	Deep and rapid, 17 mins 3 Oc gasp	32.4	Collapse Died	+	D D
26 (11.3)	B	100	7.3	7.32	9.2	0.09	0.12	5.37	120	100	36	37.0		—	2 hrs
10 c c, mor 0.01 gm phin				31 A	94.3	7.15	7.1		70 Increased amplitude 100	120 72	66, 72 deep 12	36.7		+	
				60 A	94.3	7.25		3.78	100	96	Irregular 33	36.6		+	Blood clot ting marked
10 c c and later 50 c c 0.01 gm				47 A	87.7	7.2		3.65	90	90 168 Irregular	39 18	36.1	Struggling convulsions	+	Blood very light red, nearly white or grey
				89 A	96.2	7.2		3.3	100	220	42	36.4	Struggling	+	

Acacia, 6 per Cent														
18 (8 2)	16 c c	B	100	7 0	7 08	69 7 76 9	14 8	+	Ammonia +	94 50 (18 min after injection)	66 24	37 4		
		15 A	93 4	6 9		58 6 62 2	10 5			60	130 Acceler- ated	36 7	Twitching of paws	+ Ag
		28 A	85 5	6 9	7 0	56 8 60 5	10 5	+++		60	144 Acceler- ated	36 4	Quiet	+ Ag, D
		63 A	80 0	6 8	7 0	54 9 62 3	12 5		++	60	175 36	36 2	Quiet	+ H, D, Ag
19 (13 2)	16 c c	B	100	7 2	7 14	75 78	8 5	+	+++	114 140 (5 min after injection)	75 18	37 0		None for 8 hours + in 5 min
		32 A	97	7 1		73 78	9 3		++	120	66 Shallow and irregular	36 4		H, D, Ag
		60 A	96	7 0	7 09	72 77	11 1	++	+	120	70 20 deepened	36 2		H, D, Ag
23 (9 2)		B	90 1	7 1		48 49		0 05		84 50 (4 min after acacia)	180 33			
	16 c c preceded by 40 c c sodium iodid, 21 per cent	77 A	78 4	7 0		28 36	10 5	>0 05		34	180 36}irregular 20} and 12}deepened		Collapse	+ D, Ag
		92 A	73 5	6 9		33 35	10 8	0 10		30	184 12 very deep		Collapse	+ H, D, Ag
										24	16 shallow		Died 5 min later	

TABLE 4—*Effects of Agar Sol Gel (0.1 Per Cent), Acacia (6 Per Cent and 25 Per Cent), Dextrose (18 Per Cent), Gelatin (5 Per Cent) and Horse Serum—(Continued)*

Dog No and Weight, Kg	Time of Hemo Blood globin, per Specimen, per Min	pH Colori- metric	pH Electro- metric	CO ₂ Content		Urea Nitrogen, Mg in 100 Cc	Lactic Acid, per Cent	Sugar, per Cent	Total Phos. Blood phate, Mg in 100 Cc Mercury	Pulse Rate	Respi- ratory Rate	Rectal Temper- ature, O	General Symp- toms	Sedimen- tation Rate	Aggluti- nation and Other Changes
				Per Cent by Volume	Per Cent										
Acacia, 25 per Cent															
17 (61)	B	70.4	7.2	48 50	15.0	+	None	None	74	192	48	36.4			
mor- phin	10 c c preceded by 200 c c sodium chlorid, 1 per cent, 200 c c disodium phosphate 1.1 per cent, and 75 c c sodium citrate, 2 per cent														
	9 A		7.1	35 37	13.1				106 (6 min later) 106	156 irregular	24 very 13 deep 12	35.0		+	Very D, H marked
Resuscitation with sodium bicarbonate, 9 per cent, 3 A	38 A	32.3	7.1+	34 39	13.5		None	None	100	Very irregular	Still deeper			+	Very D, H
	53 A	30.5	6.8	52 53	17.4	++			50	15	Slowed		Collapse	+	Very D, H
	3 A								0	0	Stopped		Died		
Acacia, 25 per Cent, and Dextrose, 18 per Cent															
20 (9.8)	B	84.4	7.3	54 63	11.5	++	Ammonia +++	Ammonia +++	66	200	36	37.2			
5.1 c c preceded by 16 c c per kilogram gelatin, 5 per cent, and 100 c c disodium phosphate, 5 per cent															
	31 A	62.5	7.3	48 53	11.9		++	++	70	192		37.2	Convulsions, continuous twitching	+	D
	63 A	66.6	7.25	46 51	12.5	+++	+++	+++	60			37.8	Continuous twitching	+	H, D
	78 A	66.2	7.1	44 55	13.3	+	+	+	50	200		37.6	Twitching less	+	H, D

detection, yet such a serum produces a most violent reaction and death in the same unsensitized animal from which the serum was obtained for incubation with the agar. The serum so treated by agar is not altered chemically and contains no demonstrable toxic products. However, it appears from the studies of several investigators that some change in its physical state and the minute but undetectable quantity of dissolved agar, which may occur, are the determining factors in its toxicity. Similar changes and effects are produced after treatment of serum with starch, insulin and kaolin and perhaps with other agents. Serum is not indispensable in the "toxified agar" mixture, since agar alone when properly treated so as to give the form of the sol gel described above elicits the same reactions, at least in guinea-pigs. We may now describe the toxic effects of agar sol gel in the dogs.

The results in Table 4 were obtained on one morphinized dog in a state of shock, and on two others in good condition, one of whom (Dog 26) was not morphinized. Fatal effects were produced in Dog 24 in shock and in Dog 25 in good condition at the beginning. Marked effects were produced in Dog 26, morphinized and in good condition. Shock may have been a contributory factor to the sudden death of Dog 24, but it certainly was not of Dog 25 which also died suddenly after the injection. Hence, it is apparent that it was the agar that was the determining factor in bringing on sudden death in both dogs. The tracing in Figure 1 illustrates the profound circulatory and respiratory changes occurring in Dog 25 and typifies one kind of reaction to agar. Another kind of reaction is illustrated by Figure 2, which shows several portions of a continuous tracing obtained over two and one-half hours from Dog 26.

The agar injection in this dog was not fatal within the time limit of the experiment, but nevertheless caused very marked changes in the circulation and respiration. That is, the blood pressure fell considerably immediately after the injection, then recovered to the previous level for a short time, and later fell progressively with occasional sharp falls and partial recoveries, the level reached at the end of two and one-half hours being decidedly lower than the initial level. The changes in pulse were even more marked, being characterized by slowing with a very marked increase in amplitude and some irregularity for about an hour after the injection. After that there was a recovery in amplitude and an increase in rate. The respiratory changes were characterized by irregularity in rate and amplitude. Struggling and convulsions were present during the time of circulatory collapse.

The tracing in Figure 1, which typifies the more marked reaction in the other two dogs, was obtained from Dog 25 when in good condition. It is self explanatory, and leaves no doubt that profound effects are

possible from small doses of agar. The details pertaining to the blood pressure changes and respiratory rates in all dogs are given in Table 4. Two minutes after the injection, the blood pressure from the left carotid artery fell to the zero level and showed no signs of recovery at the end of twenty minutes. After satisfying ourselves that the recording system

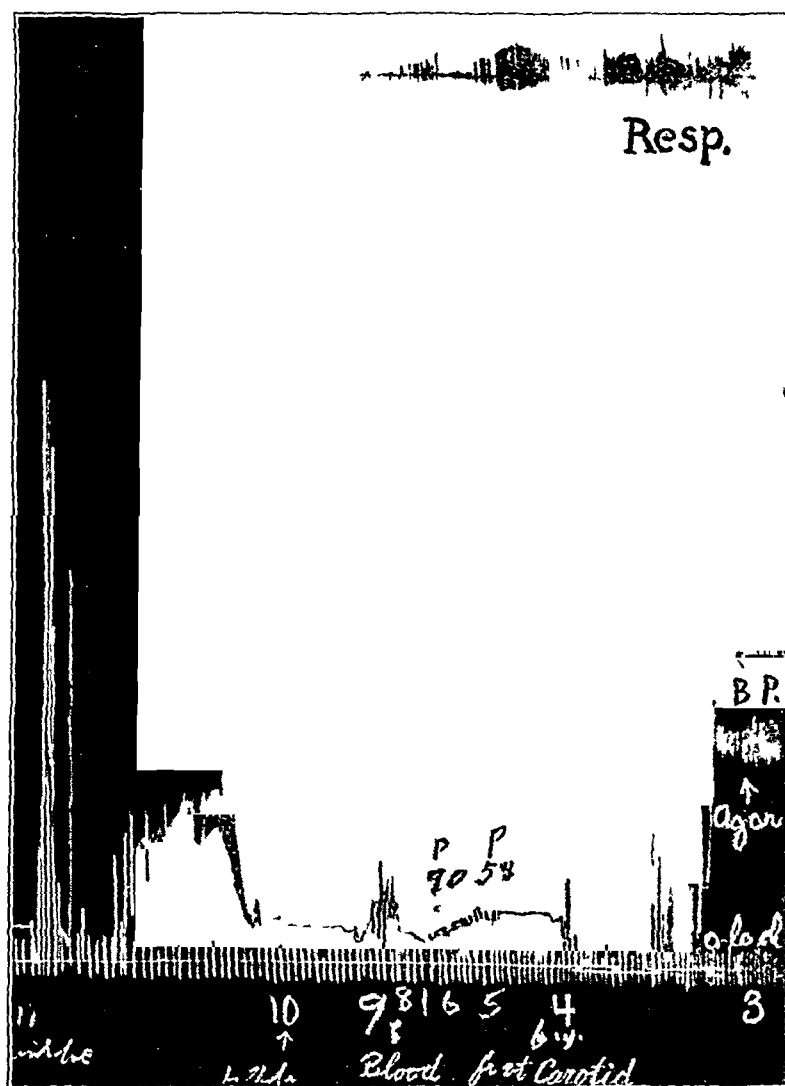


Fig 1—Effects of 0.1 per cent agar sol gel (0.011 gm per kilogram) intravenously in Dog 25, which weighed 4.4 kg. P, pulse, R, respiration, at 3, blood pressure from left carotid artery, at 4, blood pressure from right carotid artery. Blood samples were taken at 8 and 9. Resuscitation began at 10, aqueous fluid extract of mistletoe, 0.5 cc per kilogram, being administered intravenously, at 11, the same dose of mistletoe was given and in addition cardiac massage, artificial respiration and intravenous injection of 0.9 per cent sodium chlorid, but the resuscitation was unsuccessful. Time each stroke equals twenty seconds.

attached to this carotid artery was capable of transmitting changes, the attachments were switched to the right carotid artery, which revealed a blood pressure of only 16 mm, five minutes later this was reduced to the zero level. This showed that the left carotid artery had been record-

ing the pressure changes faithfully Resuscitation with mistletoe, cardiac massage and artificial respiration were tried but gave only temporary recovery, and the dog died The respiration lasted about as long as the heart continued to beat, and was very irregular in both rate and amplitude The changes in Dog 24 were essentially the same though more lasting Dogs 24 and 25 died without recognizable struggling, tremors or convulsions

As to the physical and chemical changes in the blood, these were definite and rather marked in all the dogs. Dilution was considerable, the reaction tended toward acidity (lowered p_H), carbon dioxide was reduced, and this reduction was greater than the blood dilution would

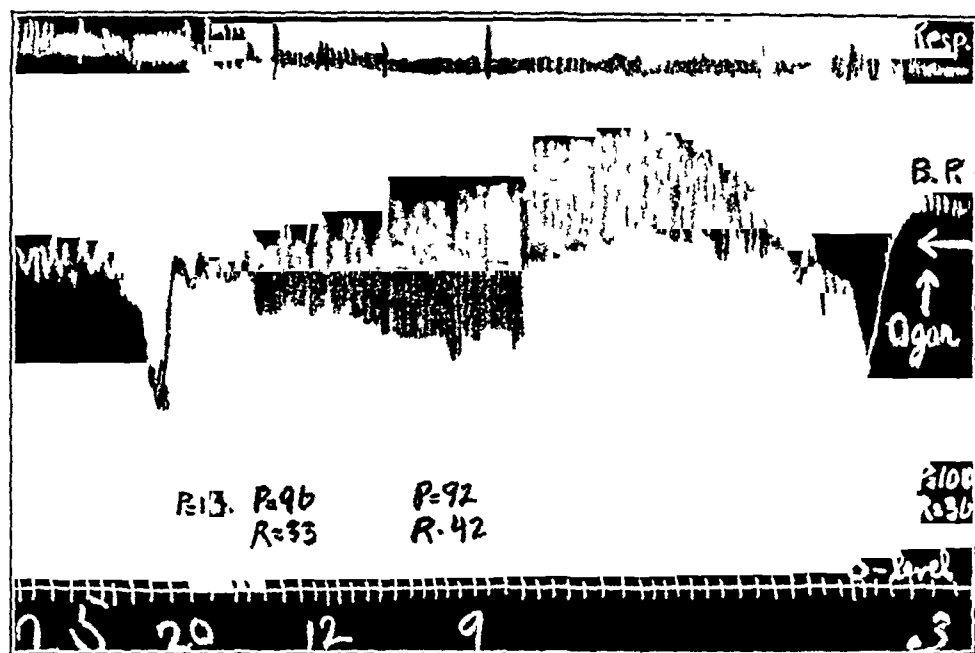


Fig 2—Effects of 0.1 per cent of agar sol gel (0.01 gm per kilogram) intravenously in Dog 26, which weighed 11.3 kg. At 3, injection of agar sol gel with effects during the next eight minutes, at 9, end of thirty-one minutes, at 12, end of fifty-three minutes, at 20, end of one hour and forty-five minutes, and at 25, end of two hours and twenty-nine minutes. Each stroke in record equals twenty seconds. The figures on the record correspond to withdrawal of successive samples of blood, the details pertaining to the respiratory and pulse rates, blood pressure and blood changes accompanying these are presented in Table 4.

indicate. The urea-nitrogen and lactic acid tended to increase in two (Dogs 24 and 26) and were lowered in one (Dog 25), while the phosphate tended to decrease in the two dogs that were tested for this. Perhaps the reason that the changes in Dog 25 do not appear more marked, or even appear opposite to those in the others, is that the animal suffered very rapid collapse and did not live long enough. The sedimentation rate of the bloods from all the dogs increased after the agar injections, and agglutination was present in all. Darkening was



Fig 3—Physical changes in arterial blood of Dog 23 after intravenous injection of 6 per cent solution of acacia, and 21 per cent solution of sodium iodid. The blood in all cases was collected under liquid petrolatum. In Tube 1, the control, no changes took place, in Tube 2, 21 per cent solution of sodium iodid, there was rapid sedimentation and darkening of corpuscles and the plasma was clear fifteen minutes after the injection of sodium iodid. In Tube 3, 6 per cent solution of acacia, there was rapid sedimentation and darkening of corpuscles, hemolysis and agglutination of corpuscles in the plasma layer thirty minutes after the acacia was given. The clear uppermost layer in each tube is liquid petrolatum.

pronounced in Dog 25 Clotting was rapid in Dog 26 and the blood appeared grayish Everything together, therefore, indicates that, despite its inertness in the ordinary sense, the intravenous injection of small doses of agar causes profound symptomatic changes and even death and definite changes in the composition and appearance of blood

Acacia, 6 and 25 Per Cent Solution—Acacia has been used in both these concentrations in human subjects, although to a greater extent in the 6 per cent concentration Its advocates have denied the possibility of deleterious effects from its use, but our results on dogs sustain those who condemn its use The results after 6 per cent acacia solution were uncomplicated by other injections in two dogs, and only by iodid in one dog, those after 25 per cent acacia solution were complicated by several other injections, although the changes following acacia agreed with those of uncomplicated injections

Taking first the results with 6 per cent acacia solution in 0.9 per cent sodium chlorid solution (Table 4), it is seen that there was considerable blood dilution in Dogs 18 and 23, a tendency toward acidity (lowered p_H) and a reduction in carbon dioxid The latter was accounted for partly by the blood dilution Lactic acid was increased in the bloods of all the animals and probably contributed, therefore, to the reduction in blood alkalinity Phosphate was not estimated, urea tended to fall, and the ammonia was variable Hemolysis, increased sedimentation rate, agglutination and darkening were present in the bloods of the three dogs These changes were rather striking, and are well illustrated by Tube 3 in Figure 3, reproduced from a Lumiere autochrome plate The increased sedimentation rate can be partly explained by the blood dilution, but dilution alone cannot explain hemolysis, agglutination and darkening The latter changes arise from the acacia Agglutination, which sometimes is accompanied by hemolysis, can be reproduced by contact of the drug with washed corpuscles in vitro, as has been shown by Karsner and Hanzlik¹ In this laboratory a rather marked darkening and peculiarly coarse appearance of the blood has frequently been observed by different workers using the acacia solution for resuscitation or for maintaining high blood pressure during experimentation on animals When such changes supervene the animals are apt to die suddenly or very soon after injection

Symptoms of twitching in the paws were present in Dog 18, in Dog 23 collapse was followed by death thirty-two minutes after injection The blood pressure fell in Dogs 18 and 23 and was only moderately raised in Dog 19, pulse rates and respiratory rates were variable Irregularity of the respiration was particularly marked The rectal temperature fell as usual in morphinized animals It can certainly be said that 6 per cent acacia solution did not act beneficially in Dog 23

with a moderately reduced blood pressure, a condition that should have been improved, according to the advocates of the solution. The effect was the contrary and the animal died, though it is true that it had received 21 per cent iodid solution (the same as is used intravenously for therapeutic purposes in patients) fifty-one minutes prior to the acacia. The effects on blood pressure were no better, of course, in Dog 18 which had not received prior intravenous injections of any kind and whose initial blood pressure was 94 mm. This indicated that it was not the iodid per se in Dog 23 that led to the fatal result but, rather, that the acacia was to be blamed.

Such results do not inspire confidence in 6 per cent acacia solution as a corrective for circulatory depression and collapse. Our experiences with acacia are in line with the unfavorable reports of Henderson and Haggard,³⁹ who compared it with other resuscitating solutions, of Richet, Brodin and Saint-Girons,⁴⁰ who tried it in hemorrhage, and of Meyer,⁴¹ who prefers modifications of Locke's or Ringer's solutions. According to Hammarsten,⁴² gum inhibits or prevents the clotting of blood, and delay in blood coagulation of dogs after acacia solutions has been reported by Foster and Whipple,⁴³ who also found increases in the nonprotein nitrogen. Nonnenbruch⁴⁴ observed increases of 100 per cent in serum protein of rabbits that had received acacia. The significance of these nitrogen and protein changes is not known, but they are at least in line with a fact hitherto insufficiently appreciated, namely, that blood is changed by intravenous injection of acacia.

Our results with 25 per cent acacia solution are complicated by those of other injections and we do not wish to overestimate them. However, we submit them for the value which they do possess. Both dogs (Dog 17, which received 25 per cent acacia solution alone, and Dog 20, which received 25 per cent acacia solution containing 18 per cent dextrose solution) had low levels of blood pressure, 74 and 66 mm of mercury, respectively, that is, they were in a condition in which the acacia solutions were indicated. Yet the ultimate results were not beneficial, since death occurred in Dog 17 and collapse became more pronounced in Dog 20, despite the fact that the blood pressures in both dogs were temporarily improved. As for the other changes, these were precisely the same as those after injections of 6 per cent acacia solution. Figure 4

39 Henderson, Yandell, and Haggard, H. W. Hemorrhage as a Form of Asphyxia, *J. A. M. A.* **78** 697 (March 11) 1922.

40 Richet, C., Brodin, P., and Saint-Girons, F. *Presse med.* **26** 581 (Nov 14) 1918.

41 Meyer, Erich. *Klin. Wchnschr.* **1** 1 (Jan 1) 1922.

42 Hammarsten-Mandel. *Textbook of Physiological Chemistry*, Ed 7, p 312, 1914.

43 Foster, D. P., and Whipple, G. H. *Am. J. Physiol.* **58** 393 (Jan) 1922.

44 Nonnenbruch. *Arch. f. exper. Path. u. Pharmacol.* **91** 218, 1921.

illustrates the circulatory and respiratory changes and Table 4 contains the details. They need not be repeated here. However, we wish to point to the rather marked decreases in p_H , i. e., tendency toward acidity of the blood, despite previous injections of alkaline phosphate and citrate in Dog 17 and of phosphate in Dog 20. Increased sedimentation rate, hemolysis and darkening of the bloods were present in both dogs, the darkening suggesting an asphyxial state which supervened in both dogs spontaneously after injection of the acacia solutions. Thus it is seen that, under the conditions, the changes produced by the 25 per cent acacia solutions were essentially the same as those produced by 6 per cent acacia solution alone. Increases in plasma protein, sugar, chlorid, urea and nonprotein nitrogen of the blood after injection of hypertonic acacia solution and glucose into normal, asphyxiated and shocked dogs have been reported by White and Erlanger⁴⁵. Hemolysis also was

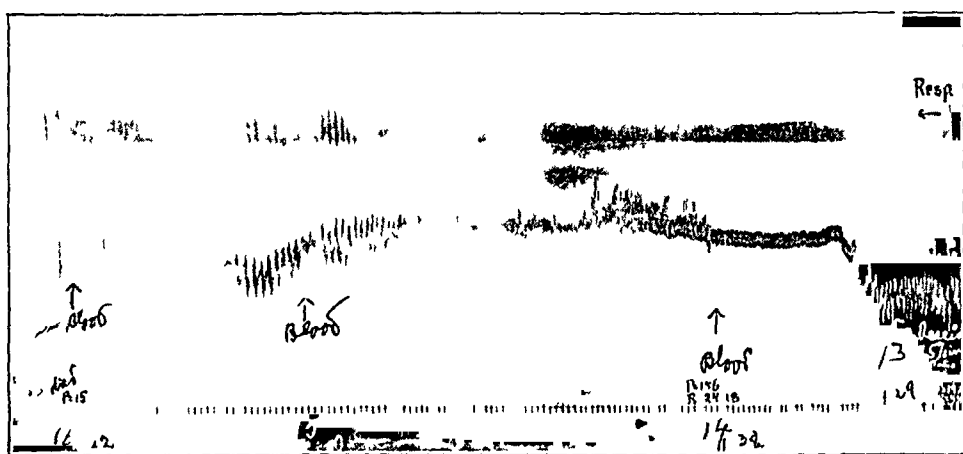


Fig 4—Effects of 25 per cent acacia solution in 0.9 per cent sodium chlorid (16 c.c. per kilogram) intravenously in Dog 17, which weighed 61 kg. P, pulse, R, respiration. Blood samples were taken at 14, 15 and 16. Each stroke in the record equals twenty seconds.

observed by these authors. They state that this was no greater than that of the controls, and that they consider the claims of Kruse⁴⁶ misleading. Our results agree with the report of Kruse. As for injurious effects from acacia solutions of different concentration and composition, the work of Erlanger and Gasser⁴⁷ indicates that the solutions as a rule did not reduce mortality but that, on the contrary, certain mixtures of acacia increased the mortality of dogs in different conditions, all of which indicates that claims of the harmlessness of injected acacia cannot be made without reservation.

45 White, H. L., and Erlanger, J. *Am J Physiol* **54** 1 (Nov.) 1920.

46 Kruse. *Am J Physiol* **49** 137, 1919.

47 Erlanger, J., and Gasser, H. S. *Am J Physiol* **50** 119, 149 (Oct.) 1919, *Ann Surg* **69** 389 (April) 1919.

Gelatin, 5 Per Cent Solution—This was tried in one dog without preceding injections. The results in Table 4 show that the changes produced were essentially the same as those from acacia. The symptoms were somewhat more marked: muscular twitching was more marked, there was increased reflex excitability, and the blood pressure fell progressively, while the pulse and respiratory rates were augmented. An increased sedimentation rate and darkening of the blood were definite, dilution was marked and lasting, and there were progressive increases in lactic acid, ammonia and urea with reduction in carbon dioxide and a tendency to increase in hydron concentration (lowered p_H). The reduction in carbon dioxide cannot be altogether accounted for by the blood dilution, and it appears that the increase in lactic acid contributed to the reduction in blood alkalinity (p_H).

Horse Serum—Ordinary normal horse serum preserved with 0.3 per cent tricresol was injected in the dosage of 16 c.c. per kilogram into Dog 21, which had previously received 200 c.c. of Tyrode's solution. The results (Table 4) show that, with the exception of less than 10 per cent of blood dilution and moderate increases in lactic acid and ammonia and a slight increase in hemolysis, the net result was beneficial. That is, alkalinity of the blood was increased to normal (p_H 7.35), the carbon dioxide remained unchanged, and the blood pressure was raised over 100 per cent, having been at the extreme low level of 20 mm. of mercury before injection of the serum. The pulse rate was slowed and the temperature fell, though no more than ordinarily occurs in morphinized animals. The urea changes were unimportant. The increase in lactic acid here was seen to occur during an increase in alkalinity of the blood, which is the opposite tendency to the acidity accompanying the injections thus far described. However, the occurrence of an increase in lactic acid during an increase in blood alkalinity is known to occur under other circumstances. Anrep and Cannan⁴⁸ have shown that the lactic acid in the blood rises when the alkalinity of the blood increases and that a fall in alkalinity is followed by a decrease of lactic acid in heart-lung preparations and spinal animals. Macleod and Knapp⁴⁹ have demonstrated an increase in lactic acid in the blood after administration of sodium bicarbonate to rabbits, and it will be seen later on in this paper that we have confirmed the increase after bicarbonate injections in dogs. This simply means that increases in lactic acid can occur under different conditions and, according to Himwich, Loebel and Barr⁵⁰ and Liljestrand and Wilson,⁵¹ an increase normally occurs during exercise. The

48 Anrep, G. V., and Cannan, R. K. *J. Physiol.* **58**: 244 (Dec.) 1923.

49 Macleod, J. J. R., and Knapp, A. *Am. J. Physiol.* **47**: 189 (Nov.) 1918.

50 Himwich, H. E., Loebel, R. O., and Barr, D. P. *J. Biol. Chem.* **59**: 265 (March) 1924.

51 Liljestrand and Wilson. *Proc. Soc. Exper. Biol.* **21**: 426, 1924.

increases during alkalinity and acidity signify alterations in cellular functions, whether these are in the musculature or in the tissues as a whole or as a result of anoxemia or asphyxial states is immaterial to the present study. For, we are primarily concerned with the demonstration of the facts of the disturbances in the physical and chemical mechanisms which, it is believed, are fundamentally concerned in the functional alterations of cells. In the case of horse serum, the minor disturbances that followed its injection apparently were not detrimental. In fact, the results as a whole indicate, if anything, an improvement in the dog's condition. The results agree in general with what is known of the relatively low toxicity of horse serum. It appears that a nontoxic serum would be a more suitable medium or vehicle for intravenous injections than such foreign agents as acacia, agar and the others to be described. For this reason, blood transfusion is to be preferred to acacia, balanced and isotonic solutions and other analogous artificial substitutes in the treatment of circulatory depression, collapse and shock.

SUSPENSIONS

The following suspensions of insoluble and particulate materials were tried: barium sulphate, 3 per cent, and fullers' earth, 0.5 per cent, both in 0.9 per cent sodium chlorid solution. The results with barium sulphate were uncomplicated and rather unexpected, while those with fullers' earth were complicated with those of previous injections. The results are presented in Table 5.

Barium Sulphate, 3 Per Cent Suspension—The intravenous injection of freshly prepared barium sulphate in a more or less gelatinous and amorphous state in guinea-pigs and pigeons causes violent reactions and death. Lumière reports similar effects in dogs after intracarotid injection toward the brain, according to him, the reactions are not elicited when the barium sulphate is in a coarsely granular state. The barium sulphate used by us was prepared according to Lumière's⁵² method, and the dosage used was the same as that used by Lumière, namely, 0.019 gm per kilogram. Three injections were made into the saphenous veins of two dogs and one injection was made into the carotid, after the manner of Lumière. Besides the usual analyses of arterial blood from the femoral artery, p_H and hemoglobin estimations in the venous blood from the right ventricle (by chest puncture) also were made. This was done in anticipation of some disparity in the circulation of the venous and arterial sides as a result of the particulate injection.

The results in both dogs were uniform. Certain changes were decidedly interesting, though negligible as far as the p_H , urea, lactic acid, sugar and phosphate are concerned. The important blood changes pertained to

52 Lumière, A. Le Probleme de L'Anaphylaxie, Paris, p. 241, 1924.

TABLE 5—Effects of Suspensions of Barium Sulphate, 3 Per Cent, and of Fullers' Earth, 0.5 Per Cent

[illegible]

* Blood from right ventricle
† Blood from left ventricle

the hemoglobin, the carbon dioxid and the physical appearance. At the end of about half an hour after injection, the arterial bloods of both dogs showed increases in hemoglobin and carbon dioxid without significant changes in p_H . A second injection in Dog 29 half an hour later again raised the hemoglobin as compared with the preceding value, but the carbon dioxid remained practically unchanged as compared with the original control values and the blood became more alkaline (p_H 7.5). The changes in hemoglobin indicate increased blood concentration, which would explain the increase in carbon dioxid in part at least. The venous blood from the right ventricle of Dog 29 tended to have lower hemoglobin and p_H values than the arterial blood. The same difference existed between the ventricular venous and femoral arterial bloods of Dog 32 as far as hemoglobin was concerned but not with respect to the p_H . The bloods of both dogs showed an increased sedimentation rate, hemolysis and agglutination, and there was darkening in Dog 29. The symptoms were characterized by twitching and tremors, a moderate fall of blood pressure, temporary acceleration of the pulse, variable respiration, a slight fall of temperature in Dog 29 and an increase in Dog 32.

The intracarotid injection in Dog 32 caused two convulsions with marked increases in blood pressure, pulse and respiration and 0.4 degree C rise in temperature. The arterial blood remained concentrated from the previous intravenous injection, the carbon dioxid increased still more, and the p_H value only slightly (alkaline tendency). The p_H and hemoglobin values of the venous blood were smaller than of the arterial blood. The interpretation of the results with barium sulphate is difficult. Some disparity in the circulation of blood between the arterial and venous sides appears to have occurred, as indicated by the hemoglobin changes. However this may be, the chemical and physical changes in the blood were not pronounced and the symptoms, though definite, were only moderate. All the changes were less marked than those with similar doses of agar and some other agents described in this article. The volume of the barium sulphate suspension injected was less. However, it is not believed that this difference would explain the difference in behavior of dogs toward agar and barium sulphate. Rather it appears that a substance in order to be reactive must be in a soluble or highly dispersed state, as is more apt to be the case with crystalloids and emulsoids than with a coarse, insoluble precipitate like barium sulphate. It has been observed by one of us (F. D.) that pigeons do not react to old, standing or aggregated barium sulphate, but do so violently to the freshly precipitated and gelatinous barium sulphate. The difference is striking and is concerned solely with a difference in dispersion of the precipitate. That is, the reaction is more marked as the dispersion increases.

Fuller's Earth, 0.5 Per Cent Suspension—A dose of 0.032 gm per kilogram was injected into Dog 29, which had received barium sulphate previously. The injection was promptly fatal. The blood pressure fell precipitously from 100 to 10 mm of mercury, the pulse and respiration became greatly accelerated, and presently both stopped (end of sixteen minutes). The changes in these functions are illustrated by Figure 5. There was some dilution, a tendency to acidity (lowered p_H), a decrease in carbon dioxide and phosphate, a slight fall in sugar, and increases in urea and lactic acid of the blood. Essentially the same symptomatic changes and also prompt death occurred in Dog 28, which received double the dose of fuller's earth after an injection of arsphenamin from

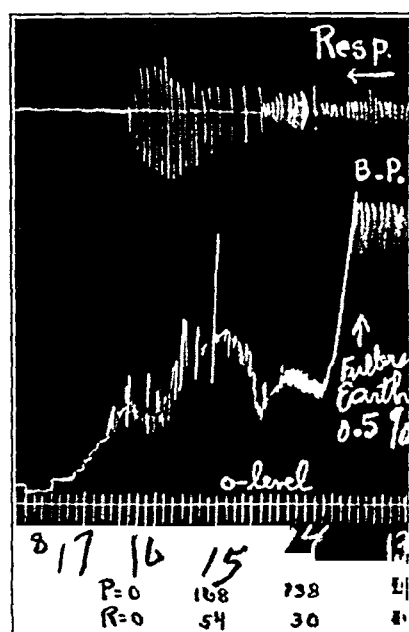


Fig 5—Effects of 0.5 per cent suspension of fuller's earth (0.032 gm per kilogram) intravenously in Dog 29, which weighed 16 kg. At 14, twitching, at 17 and 18, blood samples were taken from right and left ventricles, respectively. P, pulse, R, respiration. Each stroke of the record equals twenty seconds.

which it had partly recovered. Resuscitation was fruitless and death occurred so rapidly that no blood for analyses could be secured. From these results there is no doubt that small doses of fuller's earth are promptly fatal to dogs. When the agent is highly dispersed, it is also fatal to guinea-pigs, the effects being largely physical.

MISCELLANEOUS AGENTS

Among miscellaneous agents are included a number of salts and drugs used intravenously in human subjects and certain agents that produce violent anaphylactoid reactions in guinea-pigs. The results with all the agents in this section are presented in Table 6.

Arsphenamin—Disodium arsphenamin was prepared in the usual manner for human subjects, using the upper limit of therapeutic dosage, namely, 0.6 gm. This made the dosage 0.015 gm per kilogram for the two dogs that were observed. In one (Dog 27) the injection was uncomplicated, but in the other (Dog 28) it was preceded by peptone. However, the changes following arsphenamin were essentially the same, except for some differences in blood dilution and carbon dioxide. In the dog receiving arsphenamin alone, there was a steady increase in blood dilution to the end of 105 minutes, while in the dog that received peptone blood dilution was absent, in fact, there was some increase in concentration at the end of sixty-five minutes, as though the capillaries that had been acted on by the peptone in this dog allowed better escape of the injected solution from the circulation. In the dog that received peptone, there was a steady and considerable decrease in the carbon dioxide despite the unaltered concentration of the blood, while in Dog 27,

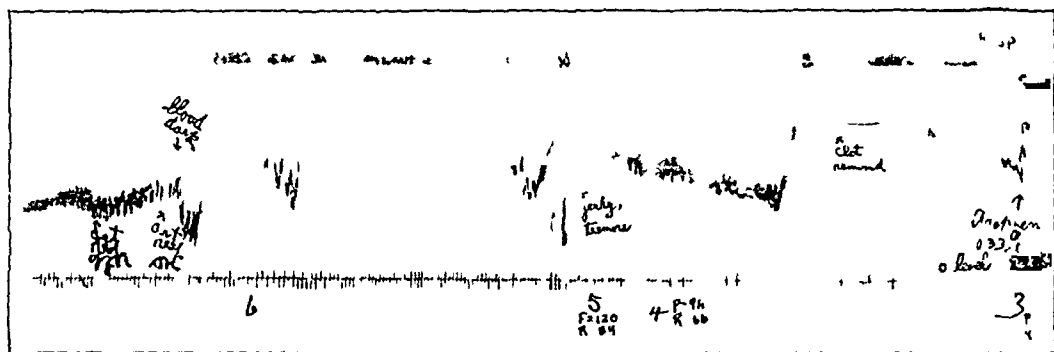


Fig 6—Effects of arsphenamin (0.017 gm per kilogram) intravenously in Dog 27, which weighed 67 kg. P, pulse, R, respiration. Blood samples were taken at 5, 6 and 7. Each stroke of the record equals twenty seconds.

which received arsphenamin alone, the carbon dioxide remained practically unchanged during one hour after administration of the drug, and only fell at the end of 105 minutes, when the blood became decidedly more diluted. With the exception of a considerable increase in p_H (7.5) at the time of some blood concentration in the dog that received peptone and arsphenamin, the p_H in both dogs at all other times fell definitely. At no time, however, did it reach neutrality or acidity, perhaps because to start with the bloods were quite alkaline. When it is borne in mind that disodium arsphenamin is itself strongly alkaline, the fall in the p_H values as a result of the injections was really considerable. Another marked effect was on the lactic acid content, which steadily increased in both dogs despite variations in blood concentration, and reached increases of about 100 per cent in both dogs at the end of one and one-half hours. Presumably this increase contributed to the reduction of the p_H . Whether the alkalinity of the disodium arsphen-

TABLE 6—Effects of Miscellaneous Agents

Dog No and Weight, Kg	Dose per Kilogram	Time of Hemo- globin, Specimen, per Min Cent	Color metric	pH Electro metric	CO ₂ Content		Lactic Acid, per Cent	Total Phos Blood Sugar, per Mg in 100 Cc Mercury		Pulse Rate	Respi- ratory Rate	Rectal Temper- ature, C	General Symp- toms	Sedimen- tation Rate	Aggluti- nation and Other Changes	
					CO ₂ Capacity	Urea Nitrogen, Mg in 100 Cc										
					Arsphenamin, 0.33 per Cent											
27 (67) ½ dose 5 cc, 0.015 gm		B	100	7.6	7.63	44 47	9.6	0.065	0.094	6.45 (2 min later	90	96 120 96	84 72 66	37.4 36.8 36.2		
		31 A	96.2	7.3	7.36	45 48	10.0	0.092	0.119	6.18	70	120	84 jerky, irregular Art resp	36.0	Tremors +	H, Ag
		60 A	92.2	7.3	7.37	45 48	9.5	0.116	0.14	5.73	70				+	H, D, Ag marked
		105 A	88.5	7.2	7.31	41 45	10.2	0.102	0.168	5.91	44		Art resp			H, D, Ag
28 (55) ½ dose 5 cc, 0.015 gm		B	101.5	7.3	7.33	51 56		0.43	0.16	4.54	70	144 180	24 30	35.2 35.2	+	Ag
		33 A	100.0	7.2	7.25	46 50		0.59	0.16	4.65	78	144	30		+ more marked	H, Ag
		65 A	109.3	7.5	7.51	45 49		0.91	0.18	4.48	80	144	30 irregular		+ still more marked	H, Ag
		85 A	99.5	7.2	7.25	43 45		0.91	0.19	4.81 accidental	40			+	H, D, Ag	
Calcium Chlorid, 9 per Cent																
18 (82)		B	98.5	6.9	7.0	51.2 54.9	13.3	++	Ammonia +	80	158	30	35.2	+		H
		24 A	67.7	6.8 or 7.0	6.74	42.1 49.5	14.8	+	+	per cent sodium chlorid, 6.1 cc per kilogram	30 0	Art resp		Died	None	H
19 (13.2) ½ dose 5 cc, 0.45 gm		B	79.0	7.4	7.27	140 140	11.5	++	+	tr	134	87	27	34.6	+	H
		35 A		7.4	7.26	96 97	11.1			7.6 and sodium bicarbonate, 15 cc per kilogram	134	49	24	34.6	+ slow	H
		60 A	90.5	7.4	7.25	96 98	11.7	+	+	(?)	130			Killed	None	H

Copper Sulphate 5 per Cent

	B†	100	7 25	61 <u>65</u>	13 3	0 146	0 107	6 18	120	24	36 8	
30 (10 2)	B*	103 6	7 25	58 —					10 S	36 after 1 min		2 con- vulsions
½ dose							(after 3 min , 70)			Deep, shal- low, stopped 48 deep	36 6	+ H
0·98 c.c., 0·049 gm	11 A	121 2	7 1	50 <u>50</u>	10 8	0 118	0 086	6 33	40			Continuous, moderate convulsions, salivation
	13 A*	112 4	7 1	55 <u>57</u>					16	Oc gasp	Died	H D no blood coagu- lation end of ½ hour

Histamin, 0.01 per Cent

26	1 B	96.2	7.2	38 46	13.3	0.114	0.124	3.3	106	220	42	36.4	+ marked
(113) No morphin	0.65 cc in five divided doses, 0.00063 gm, preceded by 20 c.c. i.g.r., 0.1 per cent												
	23 A	96.2	7.15	46 40	12.0	0.096	0.106	3.38	60	180	Irregular		+ less marked
	0.31 cc in three divided doses, 0.00031 gm												
	14 A		7.2	29 59	13.4	0.133	0.106	3.50	46		Irregular accelerated		+ most marked
	0.4 cc, 0.0004 gm												
	22 A	97.1	7.15	26 34	12.4	0.09	0.106	3.80	46	180	Deepened		+ less marked
	35 A	98.0	7.2	28 35	12.4	0.078	0.100	4.07	80		Deepened and slowed		+ most marked

TABLE 6—Effects of Miscellaneous Agents—(Continued)

Dog No and Weight, Kg	Dose per Kilogram	Time of Hemo- globin, per Specimen, Min	p_{H_2} Colori- metric	p_{H_2} Electro- metric	CO ₂ Content		Lactic Acid, per Cent	Sugar, per Cent	Total Phos Blood phate, Pressure, Mg in 100 Cc Mercury	Pulse Rate	Respi- ratory Rate	Rectal Temper- ature, O	General Symp- toms	Sedimen- tation Rate	Aggluti- nation and Other Changes
					CO ₂ Capacity Per Cent by Volume	Per Cent arsphenamin, 5 c c per kilogram									
Peptone, 5 per Cent															
27 (67) No mol phn		B	88.5	7.2	7.31	41 15	10.2	0.102	0.168	5.91	34	120 132 144	84 24 21	+	H
0.38 c c, preceded by 0.33 per cent arsphenamin, 5 c c per kilogram															
		53 A	89.3	7.65	7.65	31 37	10.5	0.180	0.156	6.32	30	126	22	+	H, Ag
		60 A	82.3	7.1	7.15	25 30	11.8	0.105	0.118	8.29	26	0	Slowed Collapse, resuscitation	+	H, Ag
28 (55) 1/2 dose		B	100	7.3	7.28	42 47		0.66	0.18	5.07 (0 min later	90 20	108 156	40 42) deep		
		30 A	105.3	7.2	7.21	52 53		0.53	0.15	4.89	90	104 141	35.2 35.2	+	Ag
		62 A	101.5	7.3	7.32	51 56		0.43	0.16	4.65	90		Irregular	+	Ag
Sodium Bicarbonate, 9 per Cent															
10 (51) No mol- phn		B	51.1	6.6	6.59	27 30	12.9				14	48	Art resp	Marked +	H
0.98 c c, preceded by 50 c c hexamethylenamin, 40 per cent, and 50 c c urea, 18 per cent															
		11 A	42.6	7.3+	7.2	92 86	10.0				30	78	Art resp	31.6	
		30 A	52.1	7.15	7.02	66 63	15.7				14	60	Art resp		
		60 A	47.6	7.15	7.0	55 62	14.5				6	48	Art resp	30.4	

	B	93	69	705	70 74	109	+	Ammonia ++	140	60	18	352	H
19 (13.2) $\frac{1}{2}$ dose 151 c.c., preceded by 6 per cent acacia, 16 c.c. per kilogram and 18 per cent urea, 100 c.c. (total)													
136 gm	30 A	80	74	737	140 140	115			140	130 75	24 30 Irregular	348	H
	66 A	79	74		120 120	113	++	+ tr	134	87	27	346	H

	Sodium Citrate, 2 per Cent												
17 (61)	B	829	72		54 58	119	+++	None	80	264	90	370	D
123 c.c., preceded by 1 per cent sodium chlorid, 200 c.c., and 11 per cent sodium phosphate, 200 c.c. 0.25 gm													
	22 A	704	72	718	52 56	109	+		deep, slowed 94	Irregular	Irregular, very rapid		Struggling Struggling
	68 A		735		45 46		++		74		Irregular, very rapid		Lighter color
	91 A		72		48 50	150	++	None	74	102	18 irregular	364	Lighter color

	Sodium Iodid, 21.7 per Cent												
13 (94)	B	840	71		56				132	100	20	354	
53 c.c., preceded by 0.9 per cent sodium chlorid, 50 c.c., and 50 per cent dextrose, 50 c.c.													
115 gm	5 A	669	70						130	88	18	352	
	20 A	729	69		53				130	81	18	352	
	65 A	735	69						112 90 (20 min later)	148	30	352	

TABLE 6—Effects of Miscellaneous Agents—(Continued)

Dog No and Weight, Kg	Dose per Kilogram	Time of Hemo- globin, per Specimen, Min	Colori- metric	pH	Electro- metric	CO ₂ Content Per Cent by Volume	Urea Mg in 100 Cc	Lactic Acid, per Cent	Sugar, per Cent	Total Phos phate, Mg in 100 Cc	Blood Pressure, Mm Mercury	Pulse Rate	Respi- ratory Rate	Rectal Temper- ature, C	General Symp- toms	Sedimen- tation Rate	Aggluti- nation and Other Changes
22 (59)		B	100	7.0	6.99	54 55 46	10.8 10.9	+	8.2	84	60	15	15	37.4	Collapse 43 min before Recovered 33 min	+	D, Ag
	8.5 cc, 1.84 gm	25 A	80	7.1	7.1	38 39	13.1	++	8.6	120 (10 min after) 100	90	24	24		None	+	D
		48 A	85.5	6.9	6.87	34 38	12.4	++	6.0	40				37.0	Severe collapse	+	D
		52 A*	76.7	7.05		38 41	12.4		8.1	20	0	Stopped	Stopped		Died		
		37 A*				39 41	12.0		9.2	0	0						
23 (82)		B	100	7.2		48 48	10.5	0.025		84	180	36	36	37.2		None	
	4.9 cc, 1.06 gm	20 A	86.2	7.15		45 51	12.0			114	180		36	37.0	Irregular	+	D, Ag
		43 A	90.1	7.1		48 49		0.05+		90			Irregular			+	D, Ag
Disodium Phosphate, 1.1 and 5 per Cent																	
17 (61)		B	88.9	7.0	7.19	34 46	12.2	++	+	Ammonia	94	110	120	37.0			
	32.8 cc, (1.1 per cent)																
	preceded by 1 per cent sodium chloride, .00 cc																
	0.36 gm	31 A	76.9	7.35	7.26	54 58	11.9			120 (7 min later) 90	264	90	90	37.0	Tremors	None	D
		51 A	82.9	7.2		54 58		+	None	86	Irregular		Irregular	36.2	Tremors stopped	None	D

20 (97)	B	89.3	6.9	6.97	56 60	13.8	+++	++	80	166	24	36.6	+ marked
	512 c.c., preceded by 5 per cent gelatin, 16 c.c. per kilogram												
	32 A	86.9	7.2	7.14	54 62	13.0	++		80	180	28	36.4	Twitching and continuous convulsions
	30 A	81.6	7.25	7.14	55 64	11.3		+	70	200	36	37.0	Twitching and continuous convulsions
	69 A	84.4	7.25		54 63	11.5	+		60				
Sodium Salicylate, 50 per Cent													
12 (102)	B	90.9	7.3	7.23	50	12.9	+		136	66	20	35.6	
	0.88 c.c., preceded by 0.9 per cent sodium chlorid, 100 c.c.												
	7 A	88.9	7.2	7.23		13.5			110	80	24 deep	35.6	Twitching + D
	17 A	79.7	7.2	7.16	40	13.1	++		126	174	28 very deep	36.4	Convulsions + D
	32 A	69.1	7.0	6.93		13.7	++		120				Collapse + D
	Postmortem from heart 6.4												
11 (60)	B	100	7.2		54 55		+		120	96	24	37.0	
	0.98 c.c., 0.19 gm												
	10 A	88.5	6.9		50 50		++		120	100	34	36.8	Twitching + D
	20 A	80.0	6.9		46 48		+++		130	100	34		Collapse + D

* Blood from right ventricle
† Blood from femoral artery

amin was responsible for the increase in lactic acid or whether it was an accompanying change of the gradual circulatory collapse that supervened is not known. The sugar tended to increase in both dogs and the phosphate remained practically unchanged. The sedimentation rate of bloods in both dogs increased and agglutination, hemolysis and darkening were present in both.

The following symptoms were observed. The blood pressure fell steadily to a shock level in Dog 27 which received only arsphenamin while it rose moderately during the course of an hour and then fell to the shock level in the peptonized dog. Figure 6 illustrates the response in Dog 27. Pulse and respiration were variable. Artificial respiration had to be used as collapse supervened in Dog 27, which received only arsphenamin. This animal also showed tremors. The rectal temperature fell in both animals.

Accordingly, higher therapeutic doses of arsphenamin produce definite and progressive blood and symptomatic changes in dogs.

Calcium Chlorid, 9 Per Cent Solution—Injections of this solution were made in two dogs, of whom one (Dog 18) had received 6 per cent acacia solution and 10 per cent sodium chlorid solution and the other (Dog 19), 6 per cent acacia solution previously. The results are presented for what they are worth. Dog 18, starting out with a lower level of blood pressure (80 mm) than Dog 19 (134 mm) died of collapse at the end of twenty-four minutes after the injection, while Dog 19 lived without marked symptomatic changes, except for slowing of the pulse and respiration. Blood dilution occurred in Dog 18, and concentration in Dog 19. The carbon dioxide of the blood was reduced in both animals out of proportion to the changes in blood concentration (volume), the increased blood carbonate after the previous injection of bicarbonate in Dog 19 being especially reduced by the calcium chlorid injection. Odaira⁵³ reports reduction of carbon dioxide in blood with isotonic calcium chlorid solution as well as with hypertonic solutions, but not with other isotonic solutions. No effect on the acid-base equilibrium of the blood in dogs after intravenous injection of from 0.5 to 1.1 gm of calcium chlorid is reported by Salvesen, Hastings and McIntosh,⁵⁴ but they state that the calcium caused an increase in phosphate. On the other hand, oral administration in dogs and man was found by these observers to produce uncompensated acidosis (p_H of 7.13 in a dog and of 7.14 in a patient with nephritis). Lactic acid was reduced in both of our dogs, and the p_H , urea and ammonia were practically unchanged. The sedimentation rate of the blood in both dogs, having been increased

⁵³ Odaira, T. Tohoku J. Exper. Med. 4: 253 (Feb. 3) 1924.

⁵⁴ Salvesen, H. A., Hastings, A. B., and McIntosh, J. F. J. Biol. Chem. 60: 277, 1924.

by the preceding injections, was retarded after the calcium. That is, the suspension of the corpuscles was more stable after calcium. Hemolysis from the preceding agents remained unchanged, as might be expected. In some respects, therefore, calcium chlorid given intravenously was not detrimental, though it is true that one of the dogs suffered fatal collapse. Both dogs had been injected previously with acacia and other agents that cause blood and symptomatic changes, as indicated in the previous sections of this paper. Certainly in Dog 19 calcium chlorid did not contribute any important detrimental changes, on the contrary, it alleviated corpuscular injury, as indicated by the improved stability of the blood in both dogs. It is interesting to note that injection of calcium chlorid into guinea-pigs does not as a rule produce anaphylactoid reactions.

Calcium salts are used therapeutically to allay or prevent certain allergic reactions and to diminish vascular permeability. The following are some recent reports on intravenous dosage and effects in human subjects. Bruhl and Buc⁵⁵ report the use of from 20 to 100 c.c. of 3 per cent calcium chlorid (3 gm. daily) in tuberculous patients with no resulting symptoms except nausea. Kawakami⁵⁶ reports the occurrence of headaches from the injection of from 5 to 30 c.c. of 1 per cent calcium chlorid solution once or twice daily. Dschalian⁵⁷ asserts that 0.1 gm. of calcium chlorid per kilogram is not dangerous. In F. von Muller's clinic at Munich, slow injections of 3 c.c. of 20 per cent calcium lactate solution were reported to cause a small pulse and weakness. Von den Velden⁵⁸ advises only small doses of 5 c.c. of 1 per cent calcium chlorid solution. Haffner⁵⁹ states that 20 c.c. of 3.4 per cent calcium chlorid solution can be injected for twenty minutes with minor effects, that 3 c.c. of 3.4 per cent solution (0.004 gm. per kilogram) causes a fall of blood pressure and respiratory stimulation in half a minute, and that 0.07 gm. per kilogram causes respiratory and cardiac standstill.

Copper Sulphate, 5 Per Cent Solution—Very small doses of this agent produce violent reactions in guinea-pigs and also in dogs. In Dog 30 the injection was uncomplicated by previous injections, but in Dog 32 there were previous injections of barium sulphate given intravenously and intracarotidly. However, Dog 32 was in good condition at the time of the copper sulphate injection and the results from both dogs agreed remarkably well. The blood changes in both dogs were interesting and profound in certain directions considering the rapid onset of death (in seven and thirteen minutes). The blood concentration increased in Dog 30, and the increase from barium sulphate in Dog 32 became somewhat reduced, though not to normal, the hemoglobin remaining about 5.5 per cent above normal. The p_H and carbon dioxide were considerably reduced. The urea, lactic acid and sugar also were

55 Bruhl and Buc. *Compt. rend. Soc. de biol.* **74** 880, 1913.

56 Kawakami. *Tokyo med. Wchnschr.*, p. 2572, 1913.

57 Dschalian. *Dissertation*, Munich, 1913.

58 Von den Velden, R. *Therap. Monatsh.* **27** 685, 1913.

59 Haffner. *Arch. internat. de pharmacol.* **23** 37, 1913.

reduced, but not as much as the carbon dioxide. Phosphate was variable. These changes cannot be explained by the change in blood volume as indicated by the hemoglobin changes. The reduction in carbon dioxide and lactic acid cannot explain the decided tendency toward acidity (reduced p_H), and the phosphate was increased in one and reduced in the other dog. The bloods of both dogs showed an increased sedimentation rate and hemolysis, darkening in Dog 30 and agglutination in Dog 32. No coagulation of the bloods of both dogs occurred at the end of half an hour. They were not observed beyond that period.

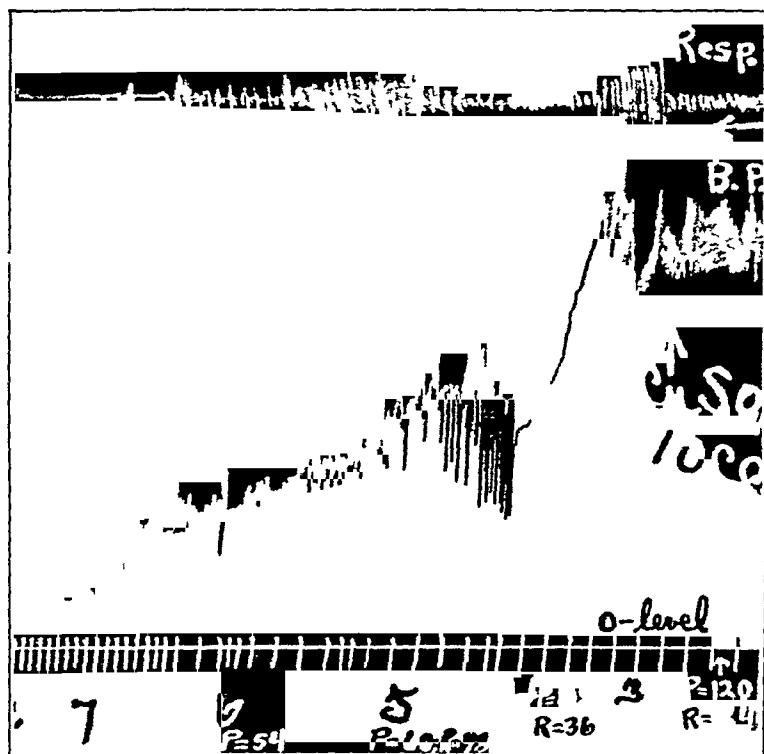


Fig 7—Effects of 5 per cent solution of copper sulphate (0.049 gm per kilogram) intravenously in Dog 30, which weighed 10.2 kg. P, pulse, R, respiration. Blood samples from femoral artery were taken at 6 and 7 and from the right ventricle at 8. Each stroke in the record equals twenty seconds.

The symptoms in the two dogs were similar. The blood pressure fell rapidly and progressively to the zero level, and the pulse and respiration were slowed. In Dog 30 the respiration stopped after the heart stopped, while in Dog 32 heart and respiration stopped together. Figure 7 illustrates the circulatory and respiratory changes in Dog 30. Convulsions were present in both animals, and in Dog 30 there was in addition considerable salivation.

It is concluded that copper sulphate produces marked blood and symptomatic changes in dogs.

Histamin, 0.01 Per Cent Solution—This was tried in an unmorphinized dog (Dog 26) which had previously received 20 c c of 0.1 per cent agar solution. Several divided doses were injected. The blood changes consisted of a slight though definite and progressive increase in concentration, fluctuation of the p_{H} between 7.2 and 7.15, an increase in carbon dioxide after the first five doses of the drug but thereafter a progressive and marked decrease to the end of the experiment, and definite decreases in urea, lactic acid and sugar. All the decreases occurred despite the slight tendency to increase in blood concentration (or reduced volume). On the other hand, the total phosphate progressively increased somewhat more than could be accounted for by the increased blood concentration. At times the sedimentation rate of the blood was increased, and hemolysis was present at the end of the experiment. There were no other symptoms besides a steady and marked fall of blood pressure from 106 to 46 mm of mercury at the end of about an hour after the first five doses of histamin, then the pressure rose to 80 mm at the end of about ninety-four minutes. The pulse was slowed and the respiration was irregular, being slowed and deepened and accelerated at different times. The concentrating effect of histamin on the hemoglobin of the blood has been reported by Underhill and Kapsinow⁶⁰. The number of corpuscles also tends to increase. These changes might be expected on account of the relaxing effect of histamin on the capillaries with the increase in their permeability asserted by some investigators. Increased vascular permeability with escape of plasma might explain the diminutions in carbon dioxide, lactic acid and sugar, but not the increase in phosphate and the fluctuating p_{H} . On the other hand, the decreases in carbon dioxide and lactic acid appeared to be greater than the degree of increased vascular permeability as indicated by the relatively small increases in hemoglobin or blood concentration. Nevertheless, the one experiment made by us indicates that under the conditions histamin definitely altered the blood chemically with respect to some important constituents. The circulatory changes were of the usual kind.

Peptone (Witte) 5 Per Cent Solution—Intravenous injection of this agent in dogs is known to produce shock somewhat analogous to that of histamin, but the results are variable. A high dosage usually is necessary. We used about one-tenth the dose frequently used for production of shock. Dog 27 received a previous injection of arsphenamin and its blood pressure was at shock level in the beginning. The injection in Dog 28 was uncomplicated by previous intravenous injections. Many of the blood changes in the two dogs were opposite, others were similar. Both dogs responded to a slight increase or practically no change in blood concentration at the end of half an hour after the

60 Underhill, F. P., and Kapsinow, R. *Am J Physiol* **63** 142 (Dec.) 1922

injections, and both showed a tendency to dilution at the end of an hour. The previously arsphenaminized dog (Dog 27) showed considerable reduction in p_H and carbon dioxide, a small decrease in sugar, a temporary increase in urea and a progressive increase in total phosphate. The marked tendency toward acidity (reduced p_H) at the end of one-half an hour might be explained by the unchanged and slightly decreased blood concentration. On the other hand, in Dog 28, the p_H was only slightly and temporarily lowered, the carbon dioxide was increased and the lactic acid, sugar and phosphate were decreased. The increase in carbon dioxide might explain the reduced p_H value at the end of one-half an hour, and yet the increase in carbon dioxide could hardly be accounted for by the slight increase in blood concentration (reduced blood volume). Although the blood changes in the two dogs are not consistent, it appears that considerable modification of the blood occurred. This is sustained by the increased sedimentation rate and agglutination which occurred in the bloods of both dogs. The hemolysis of arsphenamin in Dog 27 did not appear to be increased after the peptone injection.

The blood pressure in Dog 28, which received only peptone, remained unchanged but the pulse and respiration were slowed. In the previously arsphenaminized dog (Dog 27), the blood pressure fell about 23 per cent, the pulse was accelerated and respiration was slowed. No other symptoms were observed. It appears that peptone can produce considerable changes in the blood in the absence of marked symptomatic changes.

Sodium Bicarbonate, 9 Per Cent Solution—Injections of this solution were made in two dogs that had received intravenous injections of other solutions previously. As a result of the previous injections there was considerable circulatory depression in both dogs to start with. The bicarbonate injections resulted in temporary improvement of their conditions. The improvement was more marked and lasting in Dog 19, which had previously received 6 per cent acacia solution and 18 per cent urea solution and whose original blood pressure was 60 mm of mercury, than in Dog 16, which had been previously injected with 40 per cent hexamethylenamin and 18 per cent urea solution and whose pressure was 14 mm. The principal changes consisted of a moderate blood dilution, a marked increase in p_H , i. e., alkalinity, a marked increase in carbon dioxide and some increase in urea. Lactic acid was found to be definitely increased in Dog 19 (the only one tested for this). This was in agreement with the report of Macleod and Knapp,⁴⁹ who found an increase in lactic acid of the urine and blood after administration of bicarbonate to rabbits. Ammonia was reduced in the same dog. The increased sedimentation rate of the blood produced by other agents in Dog 16 was reduced by the bicarbonate. The hemolysis caused by the

previous agents was not altered as might be expected. The pulse and the respiration were accelerated in both dogs. The original low level of blood pressure in Dog 16 was considerably raised for half an hour, but at the end of one hour it fell to 6 mm, while in Dog 19, the pressure remained practically unchanged during the same period.

Apparently, bicarbonate is a good corrective for tendencies toward blood acidity, since the p_H of the blood of Dog 16 was raised from 6.6 to 7.3 and then to 7.15, and of Dog 19 from 6.9 to 7.4 at the end of an hour. Simultaneously, the symptoms in these dogs injured by previous injections were improved, at least they were not made worse, which was the result of the intravenous injection of several other apparently non-toxic and inert agents in this paper. The beneficial results of sodium bicarbonate observed by us agree with previous reports of others on the benefits of this salt in collapse conditions, acidotic tendencies, etc. For purposes of this study, the results with bicarbonate furnish a contrast to those with other salts and crystalloids injected intravenously. As far as p_H and carbon dioxide are concerned, they may be regarded as furnishing a proof of the correctness of the analytic data of all other agents and, in the same sense as asphyxia, they were used on occasion to test the analytic methods employed.

Sodium Citrate, 2 Per Cent Solution—Citrate was injected into a dog that had received 1 per cent sodium chloride solution and 1.1 per cent disodium phosphate solution. At the time of the citrate injection, the dog was somewhat depressed, as indicated by the level of 80 mm of mercury blood pressure and hemoglobin of 82.9 per cent. The citrate caused some further dilution of the blood without important changes in p_H , a decrease in carbon dioxide that could be accounted for largely by the blood dilution, reduction in lactic acid as compared with the control blood, absence of ammonia, and variable urea. Immediately after the injection there was some struggling, but there were no tremors or convulsions. The blood pressure rose from 80 to 120 mm and then fell to 94 mm at the end of twenty-two minutes, and then to 74 mm at the end of ninety-one minutes. The pulse rate was slowed, but respiration was very rapid and irregular soon after injection, remaining irregular though slowed at the end of ninety-one minutes. The citrate bloods appeared lighter in color than the control, which had been darkened from the previous injections. With the exception of some blood dilution and reduction in carbon dioxide, the changes produced by the citrate in this dog, that had received previous intravenous injections and was not in the best condition were relatively unimportant. Further injections were not tried.

Sodium Iodid, 21.7 Per Cent Solution—Solutions of sodium iodide of about this strength (from 20 to 21 per cent) are advocated for intra-

venous use in human subjects, the dosage varies. The 21.7 per cent solution is approximately equimolecular with 10 per cent sodium chlorid solution and is distinctly hypertonic. In Dogs 22 and 23 the injections were uncomplicated with other intravenous injections, but Dog 23 received previous injections of 0.9 per cent sodium chlorid solution and 50 per cent dextrose solution. The blood and symptomatic changes were quite uniform in all the dogs.

Taking the blood changes first, there was considerable blood dilution, a tendency toward acidity (lowered p_H), and a reduction in carbon dioxide more or less proportional to the blood dilution. In Dog 23 there was some recovery from blood dilution at the end of forty-three minutes and with this an increase in carbon dioxide to the previous percentage by volume. Lactic acid and urea were definitely increased in the two dogs receiving iodid alone, while the phosphate of one of these (Dog 22) remained practically unchanged. Sugar was not estimated. Conway⁶¹

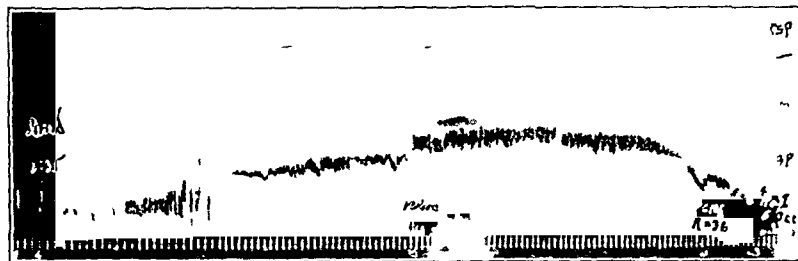


Fig 8—Effect of 21.7 per cent solution of sodium iodid (184 gm per kilogram) intravenously in Dog 22, which weighed 59 kg. *P*, pulse, *R*, respiration. Blood samples were taken from femoral artery at 5 and from heart at 7. Each stroke in the record equals twenty seconds.

has reported that sodium iodid as well as urea, sodium bicarbonate, disodium phosphate and sodium sulphate in isosmotic concentrations with blood and in the same quantities (30 c c) caused a marked fall of dextrose of the blood and total output in the urine of rabbits receiving dextrose injections. This was not true of isotonic sodium chlorid. In the two dogs observed by us, the increases in lactic acid apparently contributed to the lowering of the p_H . Increased sedimentation rate, agglutination and darkening of the arterial bloods were present in the two dogs receiving iodid alone. The records for Dog 13 were incomplete.

The blood pressure rose temporarily in Dogs 22 and 23 which received iodid alone, and then fell gradually, resulting in the collapse and death of Dog 22. In this dog the respiratory and pulse rates were accelerated during the increase in blood pressure, but in the other dog they remained unchanged, though the respiration was irregular. In Dog 13, which had previously received other solutions, the blood pressure

61 Conway, E. J. *J. Physiol.* 58: 234 (Dec.) 1923.

fell gradually from 132 to 90 mm at the end of sixty-five minutes, and the pulse and respiratory rates were slowed until blood pressure fell considerably (112 and 90 mm), they were then accelerated markedly. As all the dogs were morphinized, the temperature fell. No other symptoms such as tremors or twitchings were recognizable. Nevertheless, one (Dog 22) receiving iodid alone died from the injection. There is no doubt, therefore, that 21.7 per cent iodid solution in the doses used causes definite and considerable blood and symptomatic changes in dogs, and that these cannot be regarded as beneficial.

Disodium Phosphate, 1:1 and 5 Per Cent Solution—The 1:1 per cent phosphate solution was injected into Dog 17, which had previously received 200 c.c. of 1 per cent sodium chlorid solution intravenously, and the 5 per cent phosphate solution was injected into Dog 20, which had been previously injected with 5 per cent gelatin. At the time of the phosphate injections, both dogs had blood pressures below normal and their bloods were moderately diluted. The changes in the two were, however, in the same direction. As a result of the phosphate injection the bloods were moderately diluted and the p_H increased from neutrality (6.9 to 7.0) to alkalinity (7.35 and 7.2). In Dog 17 the carbon dioxide was considerably increased, while it remained practically unchanged in Dog 20 (the carbon dioxide capacity being increased in Dog 20), urea fell slightly, and lactic acid and ammonia were reduced. Darkening of the blood occurred in Dog 17, the marked increase in sedimentation rate of blood in Dog 20 was prevented, and the increase was absent in Dog 17. With the exception of slight blood dilution, the changes in the blood appeared to be beneficial. As to the considerable increase in alkalinity, the effect was similar to that from bicarbonate, but the increase in carbon dioxide was much less.

The symptoms in Dog 17 consisted of tremors of fifty-one minutes' duration, in Dog 20 of twitching and continued convulsions lasting sixty-nine minutes. Immediately after the injections, the blood pressure rose considerably in both dogs, at the end of about half an hour it returned to the previous level, and then gradually fell during the tremors and convulsions. In Dog 17 the pressure fell only from 90 to 86 mm at the end of fifty-one minutes, while in Dog 20 it fell from 80 to 60 mm at the end of sixty-nine minutes. In these dogs the sustaining effect of convulsions on the blood pressure often found with strychnin and asphyxial convulsions was not evident. The pulse was accelerated in both dogs, in Dog 17 respiration was slowed and irregular, in Dog 20, moderately accelerated. The rectal temperature continued to fall in Dog 17, it rose in Dog 20, presumably (in part at least) because of the convulsions. Symptomatically, therefore, the disodium phosphate solutions produced definite effects. So far as they were determined, the

blood changes were in the direction of normality. No estimations were made of phosphate, sugar and calcium. The calcium ion might be important with phosphate and citrate.

According to Binger,⁶² who has made such estimations in dogs, the calcium content of blood is decreased after alkaline and acid phosphate injections, and this investigator believes that the mechanism of toxicity is intimately bound up with the serum calcium. There are other views as to the mechanism of action, and the reports also vary as to the occurrence of symptoms of tetany. Binger did not observe tetany in his experiments. Greenwald⁶³ ascribes the toxicity of various alkaline solutions of sodium salts (containing the anions Cl , $-\text{HPO}_4$, $=\text{H}_2\text{PO}_4-$, HCO_3- and $\text{CO}_3=$) to the sodium ions rather than to the alkalinity. According to Underhill, Gross and Cohen,⁶⁴ the acid and alkaline sodium and potassium phosphates injected intravenously into dogs and rabbits reduced the blood calcium and caused symptoms of tetany, while sodium bicarbonate produced tetany without lowering of calcium. Fujimaki³⁴ reports that intravenous injections from every twenty to forty minutes of 5 cc of 5 per cent solutions of monosodium and disodium phosphates in rabbits lowered the carbon dioxide capacity of the plasma whether the solutions were used with or without epinephrin, the disodium phosphate did not affect the carbon dioxide during dextrose injection. Lactic acid formation from sugar is suggested as being important in these phenomena. However this may be, our results are not sufficiently complete nor free from complications to permit any conclusions with respect to the mechanism of the increased excitability. They may be used, however, in the same sense as the results with bicarbonate, i e., as controls of the methods employed and as indicating the general correctness of the results obtained with other agents.

Sodium Salicylate, 50 Per Cent Solution—This solution is advocated for intravenous use in human subjects without adequate basis, and practically nothing is known of its effects on the blood. Collapse is known to occur from the use of salicylate intravenously. Large doses are the rule in the treatment of rheumatic fever. The minimal fatal intravenous dose for dogs is about 1 gm per kilogram (Sollmann). We used 0.49 gm per kilogram in two dogs, of whom one (Dog 12) had received previously 100 cc of 0.9 per cent sodium chlorid solution intravenously. This would be a very high dosage for man, but we were not interested in determining the scope of intravenous dosage for man, because there is no good indication for its intravenous usage therapeutically, all the ordinary effects and therapeutic benefits being promptly obtained with oral administration of the drug. We sought to ascertain what, if any, changes in the blood, etc., would occur from adequate dosage and concentration, for if no changes occurred under these conditions, it might be assumed that no, or only unimportant, changes would occur from minimal doses. Striking results were obtained. Definite changes in the blood occurred long before circulatory collapse supervened, and both dogs died suddenly and unexpectedly at the end of thirty-two and twenty minutes.

62 Binger, C. J. *Pharm & Exper Therap* **10** 105 (Aug) 1917

63 Greenwald, I. J. *Biol Chem* **54** 285 (Oct) 1922

64 Underhill, F. P., Gross, E. G., and Cohen. J. *Metabol Res* **3** 679, 1923

The symptoms consisted of twitching in both legs, in Dog 12 these were accompanied with a few mild convulsions or tremors, but these soon disappeared. In Dog 12 about seven minutes after the injection, the blood pressure fell about 20 per cent, then it increased about 16 per cent. At the end of seventeen minutes, it was 126 mm of mercury. It fell again slightly (being 120 mm) at the end of thirty-two minutes, and two minutes later the heart stopped and the pressure fell to the zero level. The pulse rate had increased from 66 to 174 just before the heart stopped, and the respiration, which was considerably deepened, increased from 20 to 28. The temperature fell slightly just before the heart stopped. In Dog 11, the blood pressure rose from 120 to 130 mm at the end of twenty minutes, when it suddenly fell to the zero level as the heart stopped. The pulse rate was accelerated from 96 to 100 and the respiration from 24 to 34 just before the heart stopped without warning. The temperature fell only 0.2 degree. The sudden and fatal collapse that unexpectedly supervened in both dogs was rather striking.

The bloods from both dogs showed darkening and increased sedimentation rate as compared with the controls. They resembled asphyxial bloods. Other changes in the bloods were considerable, and in Dog 12 there were progressive dilution, a marked tendency to acidity (lowered p_H), a reduction in carbon dioxide more or less proportional to the blood dilution, an increase in lactic acid, and an increase in urea. Phosphate and sugar were not estimated. All the blood changes were evident while the blood pressures were high and long before fatal collapse occurred. The blood of Dog 12 postmortem had the highest acidity of any blood in this study. There can be no doubt, therefore, that 50 per cent salicylate solution in the dosage used definitely alters the blood and can cause symptoms which may result in unexpected circulatory collapse and death. Further killing of the animals for the study of smaller intravenous doses of salicylate was held unjustified.

COMMENT

The results with the different agents and solutions have been sufficiently discussed in the text and require no further discussion. They leave no doubt that a variety of agents physically and chemically different alter the blood and cause disturbances in physiologic functions. These occur with small doses and concentrations of certain agents, notably of agar. In other instances in our study, large doses and high concentrations were used though, as a rule, the concentrations correspond to those advocated for intravenous use in human subjects. With many such agents and solutions used intravenously for therapeutic purposes, the object and dosage are ill defined. Some practitioners administer large doses with the purpose of inducing marked systemic reactions

Others use small doses We have not studied the effects of small doses of the commoner agents, but this has been done recently by Handovsky⁶⁵ Handovsky reports most interesting colloidoclastic effects after small doses of 0.35 gm of sodium chlorid and of 1 gm of dextrose injected intravenously in human subjects, and on addition of these agents to beef serum in vitro The effects on both human and beef serum were practically the same, and consisted of increases in extractable cholesterol as a result of some influence (perhaps loosening up) of the salt and sugar on the colloidal structure of the serums It appears that the cholesterol of the circulating blood suffered some change in state It was also interesting that the extractable cholesterol appeared in subjects responding with physiologic disturbances as a result of the injections, and that the cholesterol increased the vascular tonus in excised organs These results of Handovsky on human and beef serums are in line with our results on changes in the blood of dogs after intravenous injections With the isotonic and hypertonic solutions of sodium chlorid, hypertonic sugar solutions, salicylate, iodid and hexamethylenamin, large doses appear to be the rule, presumably because the solutes are regarded as nontoxic, inert and harmless Our results indicate that such assumptions are unfounded despite the inertness and lack of toxicity of the solutes in the ordinary sense and when given by mouth In order to impress the uselessness and dangers of generalizations regarding disturbances or changes in vivo from inertness, etc., in vitro, we need only to point to the considerable disturbances and changes produced by agar acacia and hexamethylenamin described in the text, let alone the profound effects of more active agents and the unsuspected effects of some others The effects of agar in low dosage are so marked as to tempt one to speculate on the possible mechanism of the action involved

Agar is an emulsoid colloid possessing carbohydrate properties and is relatively inert chemically, being unreactive and nontoxic when given by mouth, but physically it is known to be rather active For instance, it agglutinates corpuscles in vitro and causes pulmonary emboli and thrombi in guinea-pigs These effects would not be expected from its ordinary properties However, in suspension and in solution, agar, like many other colloids, presents an extensive surface, and when it is introduced into the blood stream it acts as an extensive foreign surface Presumably as a result of the exposure of the blood and endothelium and perhaps of other tissues to this extensive foreign surface, changes in the surfaces of various blood elements supervene These are somewhat akin to those of the precoagulation and coagulation states leading up to agglutination, flocculation and such conditions in the blood

⁶⁵ Handovsky, H. Klin Wchnschr **3** 1354 (July 22) 1924, Munchen med Wchnschr **71** 708 (May 30) 1924, Deutsch med Wchnschr **50** 1315 (Sept 26) 1924

The latter changes have been demonstrated in corpuscles and thrombocytes with many of the agents used in this study, and are themselves indicative of surface changes. As a result of, or accompanying, these changes, there are changes in osmotic pressure, ionic equilibrium, surface tension, etc., of cell surfaces and of the cells themselves. These changes in turn determine the changes in the functional activity of the cells. There probably are other changes, and their complexity probably is greater than is realized. Adequate evidence has been obtained by one of us (F. D.) that the surface effects on the corpuscles produced by a number of the agents here reported occur from mere direct contact with the blood, and that they occur *in vitro* quite as readily as *in vivo*.

Other evidence obtained in this laboratory supports the assertion of Lumière⁵² that the reactivity of certain agents depends on the state of fineness of the particles, the reaction being more marked the finer the subdivision of particles, and mild or unobtainable with the same agent in coarse suspension. This evidence will be published in the near future. These results are cited in support of the notion of contact phenomena resulting from direct contact of agents with the blood on intravenous injection. Similar views have been expressed by other investigators, but it is believed that the results reported in this paper furnish a measure of proof that has been lacking for the assumed disturbance in physical and chemical mechanisms as a result of the contact.

Some have regarded the fundamental changes in terms of "colloidoclasia" or "hemoclasia," indicating some disruption of, or disturbance in, the colloids of the blood and tissues. Perhaps the terminology is unimportant as long as the fundamental nature of the mechanism is kept in mind for purposes of further study. Whatever the ultimate explanation of the anaphylactoid reactions and crises, the results of this paper indicate that blood and symptomatic changes occur from agents physically and chemically unrelated, some of which are relatively inert in the ordinary sense, and also that more profound changes in the blood can occur than current conceptions of regulatory mechanisms would indicate. When blood with its important normal respiratory functions for tissues in general is altered, its functions may be considerably altered, and hence also those of the tissues. This is only one reason among others why further studies along the line of this paper are needed, and it pertains especially to agents that are being injected intravenously into human subjects.

The general symptoms that supervene are no less marked than the blood changes. Variations, of course, occur. Unsuspected and sudden changes are frequently encountered. Reactions that supervene from intravenous injections occur during, or very shortly after, the injection, if at all. Therefore, our blood analyses were made over short periods after the injections. After adjustment sets in or defensive mechanisms

begin to operate efficiently, the crisis begins to pass or is unrecognizable. However, numerous possibilities exist owing to the numerous variables in the complex organism, all of which increase the difficulties of the problem. The changes in the circulation, respiration, etc., that occurred after many of the agents in this paper deserve further analysis from the physiologic standpoint. The immediate effects (for example, the sudden changes in blood pressure) may be accounted for by quite obvious and easily demonstrable gross disturbances, such as cardiac inhibition from vagus stimulation, reflex or direct, direct cardiac depression and vascular relaxation. But, of course, the ultimate changes that bring about the gross changes may still, and probably do, rest on a physical-chemical basis. Similar conceptions have been advanced not only for the action of such colloidal agents as agar but also for the actions of alkaloids, crystalloids, anesthetics and drugs in general. For this reason, studies of the character reported in this article are not only of practical interest but also of fundamental biologic importance. The bearing of the results here reported on the effects and limitations of nonspecific protein therapy and on the dangers of intravenous medication in general is obvious. These features, together with extensive summaries from the literature of objective changes of all kinds that have been reported or demonstrated in allergic reactions from various agents, have been previously dealt with by one of us⁶⁶ and need not be dealt with here. The burden of proof rests on those who assert that intravenous injections are not injurious or dangerous.

CONCLUSIONS

1 Intravenous injections in dogs of small and large doses of the following agents and solutions caused definite and important changes in arterial blood, accompanied as a rule by disturbances in physiologic functions: 10 per cent sodium chlorid solution, 85 per cent sucrose solution, 50 per cent dextrose solution, 18 per cent urea solution, 0.1 per cent agar solution, 6 per cent and 25 per cent acacia solution, 5 per cent gelatin solution, 3 per cent barium sulphate suspension, 0.5 per cent fullers' earth suspension, 5 per cent copper sulphate solution, 9 per cent calcium chlorid solution, 21.7 per cent sodium iodid solution, 50 per cent sodium salicylate solution, 0.33 per cent asphenamin solution and 5 per cent peptone solution.

2 The principal blood changes were

(a) A considerable though variable dilution immediately after injection, except after barium sulphate, peptone and histamin, which tended to cause concentration or to prevent dilution.

⁶⁶ Hanzlik (Footnote 2), *California and Western Medicine* **23** 161 (Feb) 1925

(b) A tendency to acidity as indicated by considerable lowering of p_H values, reduction from 7.3 or 7.2 to 6.9 or 6.8 being common and the lowest value observed being 6.6. Changes from the original were influenced by ether anesthesia, collapse, etc. With some agents, the changes were temporary, with others permanent.

(c) Accompanying the tendency to acidity, there usually was a reduction in the carbon dioxide (total and alkali reserve) of the plasma, and the reduction appeared to be chiefly a function of the blood dilution. Since the concentration of total carbon dioxide was reduced, the reduction in p_H was not due to carbon dioxide and presumably not to blood dilution *per se*.

(d) Of the fixed acids, lactic acid was generally increased when the p_H was lowered, and it appeared to be concerned with the tendency to acidity in part at least. Total phosphate was inconstant or variable, but the analyses were incomplete.

(e) Ammonia was frequently increased with the increase in lactic acid. Sugar showed unimportant changes, and urea remained constant or showed unimportant fluctuations even after injections of 18 per cent urea solution.

(f) Darkening of the blood together with an increased sedimentation rate, agglutination and hemolysis occurred after agar, acacia, urea, gelatin, barium sulphate, fullers' earth, arsphenamin, copper sulphate, sodium iodide and sodium salicylate, except that there was no hemolysis with iodide and salicylate and no agglutination with urea. Darkening and agglutination occurred with dextrose, darkening with phosphate and agglutination with histamin, hemolysis with 10 per cent and 0.1 per cent sodium chloride solution, horse serum and histamin, and increased sedimentation with 10 per cent sodium chloride solution. Reduction or inhibition of increase in sedimentation rate was observed with calcium chloride, sodium bicarbonate and sodium phosphate. These phenomena indicate surface changes in, and injury to, the corpuscles.

3 Disturbances in physiologic functions were indicated from changes in blood pressure, pulse and respiratory rates, ranging from moderate to profound and frequently resulting in collapse and sometimes in death. Tremors, twitching of muscles and sometimes convulsions were observed, together with occasional increases in temperature of unanesthetized dogs, salivation and diuresis. Deaths occurred from injections of 40 per cent hexamethylenamin solution, hexamethylenamin and sucrose, acacia, agar, copper sulphate, arsphenamin, fullers' earth, calcium chloride preceded by acacia and sodium chloride, peptone sodium iodide and sodium salicylate.

4 The blood and symptomatic changes were not due to the removal of small quantities of blood for analyses and were, therefore, due to

the injections themselves Induced asphxia which was used as control of the method of estimating p_H invariably lowered the p_H values (tendency toward acidity)

5 Lowering of p_H values of the blood did not occur or occurred only irregularly after the injection of 0.9 per cent sodium chlorid solution (p_H and carbon dioxide lowered in two out of five experiments) used as the vehicle or solvent for the various agents that were injected—0.1 per cent sodium chlorid solution and Tyrode's solution Increases in p_H (alkaline tendency) occurred after 1.5 and 5 per cent disodium phosphate solution, 9 per cent bicarbonate solution and 2 per cent citrate solution, though some dilution and some or no reduction of carbon dioxide (except after bicarbonate, which increased it) of the blood occurred Lactic acid tended to be augmented in alkaline bloods after the phosphate, bicarbonate and citrate, while ammonia tended to decrease Other changes in the blood after these solutions have been given in Paragraph 2 f

6 All the changes in Paragraphs 2, 3 and 5 occurred in morphinized and unmorphinized dogs, with and without artificial respiration Therefore, the blood changes appeared to be produced independently of the respiration, and resulted from direct contact of the solutions and agents with the blood and tissues

7 It is suggested that the basis of the blood and symptomatic changes that were demonstrated, and which resulted from contact with a variety of agents physically and chemically unrelated, rests fundamentally on disturbances in important physical and chemical mechanisms of the blood and tissues

8 The practical bearing of the results on nonspecific foreign protein therapy and on intravenous medication is indicated and has been discussed in previous articles

THE LIPOID PARTITION IN BLOOD IN HEALTH AND IN DISEASE *

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The development in recent years of methods for the chemical analysis of the fatty constituents of blood has made possible the study of the partition of cholesterol and lecithin between the blood plasma and cells, a matter of great interest in view of the reported antagonism existing between these substances. This article is part of a group of studies on the concentration and distribution of these lipoids in normal and pathologic blood, and the relation of these factors to certain physico-chemical properties of the red blood cells possibly dependent on them. It is also planned to study from this standpoint the balance between free and combined cholesterol. The investigation herein reported is concerned only with the lipoid partition in the blood of normal subjects and of patients suffering from a variety of conditions in which abnormalities in blood lipoids were expected.

It has been reported by Bloor,¹ Chauffard, Laroche and Grigaut,² and Gorham and Myers³ that the total cholesterol content of normal blood plasma is only slightly above that of whole blood though generally somewhat greater than that of the corpuscles. The "lecithin" content of the plasma on the other hand is, according to Bloor, approximately half that of the corpuscles. The constancy that is understood to exist in the ratio of these lipoids in plasma and cells has been regarded as suggestive of a relationship between them, one exerting a neutralizing or balancing influence against the other. The lipoid content of the cells has been investigated by Bloor in a variety of pathologic conditions and has been found to be sufficiently constant to warrant the suggestion that to avoid confusion little attention should be directed to the figures for cell lipoids.⁴

In 1912 Grigaut and L'Huillier⁵ published figures for the cholesterol distribution in forty-six pathologic cases in which the serum values varied between 71 and 840 mg per hundred cubic centimeters, while the

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1 Bloor, W R. J Biol Chem **25** 577, 1915

2 Chauffard, A, Laroche, G, and Grigaut, A. Compt rend Soc de biol **70** 336, 1911

3 Gorham, F D, and Myers, V C. Cholesterol Content of Blood, Arch Int Med **20** 599 (Oct) 1917

4 Joslin, E P. The Treatment of Diabetes Mellitus, Ed 2, Philadelphia, Lea & Febiger, 1917, p 97

5 Grigaut, A, and L'Huillier, A. Compt rend Soc de biol **73** 202, 1912

cell values remained within the limits of 110 to 195 mg per hundred cubic centimeters. The latter was regarded as normal. This constancy of corpuscle cholesterol has since been confirmed by Bloor.

Later, Henes⁶ reported normal figures for the distribution of cholesterol between serum and corpuscles, his conclusion being that 55.6 per cent of the total cholesterol is found in the serum.

The likelihood of variations in the cell concentrations of lipoids, however, was indicated by the results of various investigators. Striking increases in the proportion of cholesterol ester in the cells were shown by Knudson⁷ to take place during fat absorption. Pacini reported changes in the cholesterol distribution in anemia⁸ and in gallstones.⁹ Undernourishment induced by war diet was found by Rosenthal¹⁰ to effect diminution in the cholesterol content of the cells. The work of MacAdam and Shiskin¹¹ on the relation of splenic function to anemia has shown variations to exist in the corpuscle cholesterol. It appears evident therefore that the question of lipid distribution has by no means been definitely settled.

As Bloor points out, however, comparison of the work of various investigators is of little value unless due regard is given to the methods used, the time of drawing blood in relation to the last ingestion of food, and the lapse of time between collection of blood and analysis. A further factor of importance in this connection is the means employed for measuring the relative volume of the cells.

METHODS

The investigations of Brinkman¹² on the osmotic resistance of red blood cells would seem to indicate that washing the cells with isotonic salt solution removes some of the phosphatids. Consequently we should expect a difference between corpuscle "lecithin" values determined directly on washed cells and by calculation from the cell volume and values for whole blood and plasma. Partly for this reason and partly for its greater simplicity, we have chosen the latter procedure.

Blood was collected in oxalated tubes. Determinations were conducted as soon as possible after the blood was drawn, at least to the stage at which no alteration in lipid composition or distribution could occur. Values for cell lipoids were obtained by calculation as indicated above.

6 Henes, E. *Deutsch Arch f klin Med* **111** 122, 1913.

7 Knudson, A. *J Biol Chem* **32** 337 (Dec) 1917, *ibid* **45** 255 (Jan) 1921.

8 Pacini, A. *J P Am Med* **24** 92, 1918.

9 Pacini, A. *J P Med Rec* **94** 441 (Sept 14) 1918.

10 Rosenthal, F. *Deutsch med Wchnschr* **45** 571 (May 22) 1919.

11 MacAdam, W., and Shiskin, C. *Quart J Med* **16** 193 (April) 1923.

12 Brinkman, R. *Arch neerl physiol* **6** 451, 1922.

Cell Volume—The blood, thoroughly mixed by inversion, was drawn into the graduated capillary tubes of the Daland hematocrit and centrifugated at 3,000 revolutions per minute for three minutes. The reading was made with the aid of a hand lens, and the tubes were then centrifugated another minute. Invariably there was no change in the reading. This method is quicker and, we believe, more accurate than the use of a graduated centrifuge for such measurements.

Cholesterol—Cholesterol determinations were made by the method of Myers and Wardell¹³. In preliminary work with this method it was apparent that one-half hour extractions were not sufficient to remove completely all the cholesterol. In most cases one hour was found to suffice, so allowing a margin of safety all extractions were conducted for one and a half hours.

The development of the final color was allowed to proceed in the same light by which the readings were later to be made, as recommended by Bloor, Pelkan and Allen¹⁴. For colorimetric comparison a standard solution of cholesterol in chloroform was required in place of the artificial (naphthol green B) color standard that Myers and Wardell recommend for development of the color in the dark. It was necessary to prepare fresh standard cholesterol solutions from time to time, as the color developed from old solutions appeared more bluish than that from the chloroform extracts of desiccated blood.

Lipoid Phosphorus—The determination of lipid phosphorus was made on the alcohol-ether extract, 25 c c of which contained the lipid fraction of 1 c c of whole blood or plasma. Five cubic centimeters of the extract were filtered into a pyrex test tube graduated at 5 c c, and evaporated to dryness on the water bath. The residue was oxidized by digesting over a microburner with 0.5 c c of a mixture of equal parts of concentrated nitric and sulphuric acids, precautions being taken against overheating the tube. The oxidation took place in three distinct stages. First, the water and oxides of nitrogen passed off, then sulphuric anhydride fumes, finally, these dissipated and the oxides of nitrogen reappeared. At this juncture the heating was discontinued and, after a few minutes cooling, a drop of 1 per cent sucrose solution was introduced. The flame was again applied until the mixture just came to a boil. The sucrose solution is said to split up a refractory nitric acid-phosphoric acid complex which prevents the phosphoric acid from reacting colorimetrically. After cooling, from 1 to 2 c c of distilled water was added and the solution was again allowed to cool. To effect partial neutralization of the strong acid, 6 or 7 drops of concentrated sodium hydroxid

13 Myers, V. C., and Wardell, E. L. J. Biol. Chem. **36** 147 (Oct.) 1918.

14 Bloor, W. R., Pelkan, K. F., and Allen, D. M. J. Biol. Chem. **52** 191 (May) 1922.

was carefully introduced, the tube was again cooled and distilled water added to the 5 c c mark. For the color development the Brigg's modification¹⁵ of the Bell and Doisy¹⁶ procedure for inorganic phosphorus was used.

COMMENT ON RESULTS

Normal Cases—These included patients who were members of the hospital and laboratory staffs and some patients whose diagnosis indicated no disturbance in lipid metabolism. The latter group included five cases of psychosis and two minor surgical cases. Among the hospital cases it was our experience that the average cell volumes fell about 5 per cent below the limits usually regarded as normal, but only those

TABLE 1—*Normal Cases*

Case	Cell Vol- ume, per Cent	Whole Blood			Plasma			Cells		
		Choles- terol, Mg per 100 C c	Lipoid Phos- phorus, Mg per 100 C c	Ratio Chol Lip P	Choles- terol, Mg per 100 C c	Lipoid Phos- phorus, Mg per 100 C c	Ratio Chol Lip P	Choles- terol Mg per 100 C c	Lipoid Phos- phorus, Mg per 100 C c	Ratio Chol Lip P
1	39	183	15.2	12.04	200	11.9	16.80	156	20.3	7.68
2	37	175	14.2	12.32	203	11.3	17.96	127	17.8	7.14
3	43	160	11.7	13.67	189	9.2	20.54	121	15.1	8.01
4	42	142	12.1	11.73	155	8.8	17.61	124	16.7	7.42
5	40	140	13.3	10.52	146	9.7	15.05	131	18.8	6.97
6	45	148	11.3	13.10	169	7.4	22.84	122	16.0	7.63
7	48	146	11.7	12.48	193	8.8	21.92	96	14.8	6.48
8	43	154	15.1	10.20	169	11.3	14.96	135	20.2	6.68
9a	41.5	190	15.5	12.25	246	14.1	17.44	111	17.5	6.34
9b	42.5	165	14.0	11.79	238	11.7	20.34	66	17.1	3.86
10a	43	170	16.3	10.43	261	13.8	18.91	72	19.5	3.69
10b	45	140	13.7	10.22	199	10.4	19.13	68	17.8	3.82
10c	42	162	15.4	10.52	220	13.3	16.54	81	18.3	4.43
11	42	161	12.5	12.88	233	8.9	26.19	62	17.4	3.56
12	42	133	12.3	10.81	168	10.1	16.63	83	15.2	5.46
13	46	154	14.3	10.77	200	11.4	17.54	91	17.2	5.20
14	40	160	13.6	11.76	177	9.8	18.06	135	19.3	6.99
15	41	151	13.2	11.44	154	9.8	15.10	146	18.0	8.11
16	40	165	13.1	12.59	180	9.5	18.94	142	23.2	6.12
17	41	110	13.6	8.09	105	9.4	11.17	117	19.8	5.91
18	43	118	13.7	8.61	118	10.8	10.93	118	17.4	6.78
Averages		153.7	13.61	11.34	186.8	10.54	17.81	109.7	17.97	6.11

hospital cases were selected in which the cell volume was above 40 per cent. Perhaps the hematocrit method was responsible for the lower cell volumes in apparently normal cases.

From Table 1 it will be seen that both the cholesterol and lipid phosphorus values for normal whole blood agree in general with those recorded in the literature. While the normal range for cholesterol in whole blood using the foregoing method is given as from 140 to 170 mg per hundred cubic centimeters, occasionally values outside these limits were obtained. The figures for lipid phosphorus in whole blood range somewhat higher than those usually reported. Bloor, for example, gives 12 mg lipid phosphorus (recalculated from "lecithin" on the basis of 4 per cent phosphorus content) as the average for whole blood.

¹⁵ Briggs, A. P. J. Biol. Chem. **53** 13 (July) 1922.

¹⁶ Bell, R. D., and Doisy, E. A. J. Biol. Chem. **44** 55 (Oct.) 1920.

of men, while here the average value is 13.6 mg. However, it is in the distribution of the lipoids that these results differ from those of others.

It is said that plasma cholesterol approximates though it is slightly higher than that of whole blood. Although the plasma cholesterol values presented are in practically all instances higher than the corresponding whole blood values, they can hardly be said to approximate them nor are the increments by any means constant. In the cases analyzed, an average of 68 per cent of the total cholesterol of the whole blood was present in the plasma, as compared with from 55 to 60 per cent reported by others. In other words, instead of the plasma cholesterol values being only slightly higher than corresponding whole blood values, these data show them to be anywhere from one to one and a half times as high. Consequently the concentration of cholesterol in the corpuscles in these cases is lower than usually reported and varies over a considerable range.

Cases 17 and 18 have been included in this series to illustrate the not infrequent occurrence of the blood of a healthy individual which is apparently normal in all respects except for its low plasma cholesterol content giving rise to low blood values.

Since the corpuscle values were calculated, as described above, they are subject to wider variation and greater possible error than those values obtained directly. Incidentally the corpuscle values reported by Bloor¹ for normal males were not calculated from actually determined cell volumes but were based on the average cell volume of Keith, Rowntree and Geraghty.¹⁷ This average (43 per cent) was obtained from only eighteen normal persons, the limits (from 32.7 to 49 per cent) being obviously too extreme to warrant the use of the average in any single case.

The concentration of lipid phosphorus in cells varies within narrower limits than in plasma. According to our data it is from one and a half to two times the plasma concentration, as compared to the 2:1 ratio obtained from Bloor's data. It is worthy of note that, except for a few instances, from 50 to 60 per cent of the total lipid phosphorus of whole blood is contained in the cells.

In the majority of the normal cases tabulated the ratio of cholesterol to lipid phosphorus in the cells averaged about 6:1. Several cases showed ratios somewhat lower than this on account of diminished concentrations of cholesterol. The plasma ratios of cholesterol to lipid phosphorus were less constant and averaged about 17:8. In terms of lecithin this means that there is somewhat more lecithin in the plasma than cholesterol. Bloor's data indicates the opposite, but it must be remembered that cholesterol values determined by his method are considerably higher than those determined by the method of Myers and

¹⁷ Keith, N. M., Rowntree, L. G., and Geraghty, J. T. *Arch. Int. Med.* 16: 547 (Oct.) 1915.

Wardell In normal whole blood the ratio of cholesterol to lipid phosphorus was quite constant, averaging around 11.3. Those cases referred to above of normal blood with relatively low plasma cholesterol content showed the only marked discrepancy in this respect.

TABLE 2—*Pathologic Cases*

Case	Cell Vol ume, per Cent	Whole Blood			Plasma			Cells		
		Choles terol, Mg per 100 C c	Lipoid Phos phorus, Mg per 100 C c	Ratio Chol Lip P	Choles terol, Mg per 100 C c	Lipoid Phos phorus, Mg per 100 C c	Ratio Chol Lip P	Choles terol Mg per 100 C c	Lipoid Phos phorus, Mg per 100 C c	Ratio Chol Lip P
Uremia										
51	20	280	20.2	13.86	346	19.4	17.83	15	23.5	0.66
52	20	185	13.7	13.50	184	11.0	16.73	100	24.5	7.76
53	18	300	18.1	16.58	354	19.5	18.15	56	11.7	4.79
54	22	200	12.9	15.50	196	11.9	16.47	214	16.4	13.05
55	9	225	15.7	14.33	237	11.9	19.91	100	54.4	1.84
56	35	147	14.1	10.43	171	11.3	15.13	103	19.4	5.32
56a	36	135	14.2	9.51	163	10.8	15.9	86	20.3	4.24
57	37	120	12.2	9.83	135	9.9	13.63	95	16.2	5.86
58	18.5	130	10.8	12.04	127	9.0	14.11	140	18.9	7.41
59	13	153	13.3	11.50	155	11.4	13.59	139	26.1	5.32
Diabetes										
60	38	172	15.4	11.16	212	11.9	17.82	108	21.1	5.12
61	49	150	19.0	7.90	215	15.9	13.52	82	22.2	3.69
62	36	134	13.1	10.23	160	9.8	16.33	89	26.7	3.33
63	43	182	15.2	11.97	274	13.2	20.76	60	17.9	3.35
64	33	150	14.9	10.06	171	12.0	14.25	106	20.9	5.07
65	42	140	13.5	10.37	133	10.2	13.04	150	18.1	8.29
66	40.5	161	16.4	9.82	175	11.4	15.35	141	23.7	5.95
67	34.5	142	11.7	12.13	120	8.1	14.81	182	18.5	9.83
68	23	651			869			0		
Pregnancy										
69	32	207	16.7	12.39	230	16.1	14.28	159	18.1	8.78
70	35	222	19.0	11.68	245	15.7	15.60	180	25.2	7.14
71	30	225	17.9	12.56	300	22.5	13.33	50	7.0	7.14
72	33.5	242	16.6	14.58	283	22.6	12.52	161	4.8	33.54
73	30	175	15.9	11.01	200	13.3	15.04	117	20.3	5.76
74	33.5	230	17.8	12.92	285	16.9	16.86	119	19.7	6.04
Pernicious Anemia										
75	25	103	11.9	8.66	100	9.5	10.53	112	19.2	5.83
76	16	138	11.2	12.32	130	8.8	14.77	181	23.8	7.61
77	19	78	13.1	5.95	65	9.7	6.70	132	27.6	4.78
78	9	116	9.4	12.34	107	7.4	14.46	211	30.0	7.03
Banti's Disease										
79a	13	127	11.9	10.67	117	8.5	13.76	192	34.6	5.55
79b	16.5	151	14.5	10.41	131	11.7	11.20	254	28.4	8.94
80a	17.5	138	12.4	11.13	133	9.3	14.30	160	26.8	5.97
80b	23	120	12.6	9.52	95	7.8	12.18	204	28.7	7.11
81	15	150	13.3	11.28	156	9.4	16.60	113	35.2	3.22
Jaundice of Infectious Disease										
82	40	130	16.2	8.02	97	11.3	8.58	180	23.5	7.66
83	31	185	18.3	10.11	205	12.2	16.80	139	31.9	4.36
84	39	170	19.4	8.76	150	22.6	6.64	201	11.4	13.96
85	21	140	19.0	7.37	122	19.2	6.35	205	18.1	11.32
86	19.5	113	8.6	13.14	70	5.3	13.21	292	22.0	13.27
87	36	111	12.8	8.67	88	9.0	9.78	153	19.4	7.88
Obstructive Jaundice										
88	16	125	17.3	7.22	107	16.1	6.64	219	23.8	9.20
89	21	128	10.6	12.07	110	11.5	9.57	195	7.2	27.08
Cholelithiasis										
90	45	136	19.3	7.05	165	17.5	9.43	100	17.2	5.81
91	38	225	21.5	10.46	250	20.6	12.13	184	22.9	8.04

From these data it would appear difficult to assume a constant balance to exist between the amounts of cholesterol and lecithin in the blood and to accept the suggestion of an antagonism based on a quantitative relation between these substances.

Uremia—The hypercholesterolemia encountered in some uremic cases may result from either an increase in both plasma and cells or low cell volumes accompanying normal or increased plasma values. In the plasma of these cases, the ratio of cholesterol to lipid phosphorus is normal, but in those uremic cases with hypercholesterolemia a distinct disturbance in the absolute as well as the relative amounts of the lipid substances is noted in the cells. Both cholesterol and phospholipoid vary widely and independently of each other.

Taking into account the varying degrees of anemia present, the fraction of total whole blood cholesterol and lipid phosphorus contained in the plasma of these uremic cases is, on the average, above normal, in spite of the higher concentration sometimes encountered in the cells.

Bloor's¹⁸ observations indicate an increase in fat and lipid phosphorus in the corpuscles of nephritic patients, which he regards as due to the retarded assimilation accompanying the general metabolic disturbance associated with lowered alkali reserve. In agreement with the observations of others, we have found no relation to exist between the hypercholesterolemia of nephritis and the degree of nitrogen retention or carbon dioxide combining power. In their recent paper on fat metabolism in nephritis, Hiller, Linder, Lundsgaard and Van Slyke¹⁹ suggest that the failure of the mechanism for transporting lipoids from the blood to the tissues may account for their accumulation in the blood. Whatever the causative factor of nephritic lipemia may be, however, it appears to effect not merely a piling up of fats but also a disturbance in their customary distribution.

Pernicious Anemia—The hypocholesterolemia in these cases results from a diminution in concentration in the plasma with normal or even increased values in the cells, the latter having little influence on whole blood figures owing to the low cell volumes. There is evident in some cases a tendency toward increased concentrations of lipid in the cells in pernicious anemia, although the proportion of the total whole blood lipoids found in the cells is below normal.

It may be remarked at this point that a tendency toward the accumulation of lipoids in the corpuscles is observed in most cases exhibiting anemia to a marked degree whether primary or secondary in origin. This would seem to indicate the existence of some relation between the lipoids of the cells and fat metabolism. It is suggested that the fat transport mechanism may be disturbed to the extent of placing too great a load on the relatively few blood cells. The corpuscles might be said to be overworked in their attempt to convert fatty acids to lecithin. Horiuchi,²⁰ however, failed to observe increased lecithin values in the

18 Bloor, W. R. J. Biol. Chem. **31** 575 (Sept.) 1917

19 Hiller, A., Linder, G. C., Lundsgaard, C., and Van Slyke, D. D. J. Exper. Med. **39** 931 (June) 1924

20 Horiuchi, Y. J. Biol. Chem. **44** 363 (Nov.) 1920

corpuscles of rabbits in experimental anemia and suggested that the resultant lipemia was due to the limitation of this function of the corpuscles

These data do not agree with the statement of Bloor and MacPherson²¹ to the effect that the blood lipid values in anemia are normal so long as the percentage of corpuscles is more than one half the normal value

It is well to recall, in discussing anemic blood, that the error in calculation of cell values increases in inverse ratio to the cell volume

Banti's Disease —The first two cases of Banti's disease studied manifest a divergence from the normal cholesterol partition in that there is a diminution in the plasma accompanied by a considerable increase in the cells, though because of the marked anemia whole blood values are low MacAdam and Shiskin¹¹ report similar results on a case of Banti's disease The lipid phosphorus content of the cells in these cases is also abnormally high

In each of these cases splenectomy was performed, following which the cholesterol lipid phosphorus ratios dropped in the plasmas and rose in the cells, a decided absolute increase of cholesterol taking place in the cells of both cases While the plasma cholesterol rose in only one of the cases, the plasma fraction of whole blood cholesterol fell in both cases The second series of analyses were made one day after operation The authors mentioned above have shown that the rise in plasma cholesterol following splenectomy is gradual and may even be preceded by a slight fall We have noticed this diminution in phospholipoid as well as in cholesterol in Case 80 This, however, may have been related to an accompanying cirrhosis of the liver, which was the probable cause of death in this case It may be recalled that Bloor mentions low vitality as a condition manifesting hypocholesterolemia

The third case did not come under our observation until the seventeenth day after splenectomy was performed The cholesterol partition is normal, though the lipid phosphorus of the cells is quite increased

Pregnancy —Six cases are tabulated showing the rise in lipoids that occurs during the later months of pregnancy The accumulation of lipoids is observed chiefly in the plasma, though in some cases, particularly in regard to cholesterol, the cells also participate Considerable variation is found to exist in the phospholipoid concentration of the cells A marked change in fat metabolism takes place, which alters the distribution as well as the concentration of lipoids in the blood In this connection we are led to recall the observation of Meigs, Blatherwick and Carey²² to the effect that the blood phosphatids are

21 Bloor, W R, and MacPherson, D J J Biol Chem **31** 79 (July) 1917

22 Meigs, E B, Blatherwick, N R, and Carey, C A J Biol Chem **37** 1 (Jan) 1919

the precursors of milk fat, the prenatal increase thus being a preparation for the impending demand

Jaundice of Infectious Disease—The most characteristic feature of the lipid picture in the blood in the type of jaundice found in infectious diseases is the distinct increase in the cell cholesterol. A diminution in the plasma cholesterol is generally observed also. The phospholipoids of both plasma and cells are usually normal, though occasional variations may occur. These changes in lipid partition are masked when determinations are made on whole blood alone.

Obstructive Jaundice—The cases of obstructive jaundice presented show a definite increase in cell cholesterol. It is a question, however, whether this may be related to the jaundice per se or to the marked anemia accompanying it. The lipid phosphorus value in the cells of Case 89 is much reduced, an unusual occurrence in anemic blood.

Cholelithiasis—The unreliability of cholesterol determinations as a means of diagnosis of gallstones is illustrated by the two cases reported, the diagnoses of which were confirmed at operation. Case 91 shows a hypercholesterolemia (whole blood) while Case 90 shows what may probably be regarded as a low normal. The partition of cholesterol is approximately normal in both cases. It is interesting to note that the lipid phosphorus values in the plasmas are increased.

Diabetes—While the whole blood concentrations of cholesterol and phospholipoid fall within normal limits, in most of our diabetic cases there is sometimes observed a distinct shift in the partition of cholesterol, usually in favor of the plasma. The cells may show increased phospholipoid values which may affect the whole blood figures, especially in a case of polycythemia like Case 61. Lipemia is not a common occurrence in the usual run of diabetic cases, especially those cases under treatment. Since the advent of insulin we have had difficulty in finding cases of diabetic lipemia. The one case given, that of a 6 year old girl with an extreme lipemia that persisted in spite of heavy doses of insulin, showed at one time 12.1 per cent ether-soluble matter in the blood.

CONCLUSIONS

1 Variations in the lipid content of normal human plasma and cells may occur over a somewhat wider range than previous work indicates.

2 Since no constant balance between cholesterol and "lecithin" appears to exist in normal blood, the theory of an antagonistic relation between these substances does not receive support.

3 Analyses of the blood in a series of pathologic cases show that considerable variation in the cell concentration of lipid substances may occur, particularly in blood of low cell content.

CONGENITAL INTRACARDIAC FISTULAS

THEIR EFFECT ON THE DEVELOPMENT OF THE HEART *

EMILE HOLMAN, M D

CLEVELAND

The abnormal development of the heart that occurs in the presence of congenital anomalies has not been well understood and, consequently, not adequately explained. The following excerpt from a recent standard textbook¹ illustrates the difficulties of explanation presented by the patent septum

In an uncomplicated patent interventricular septum the blood seeks its way through the patency, *flowing back and forth between the two ventricles*, and if the opening is small the pressure between the right and left ventricles may remain equal, giving rise to no symptoms. In the majority of cases, however, the pressure in the two chambers is unequal. Thus *part of the blood is left over during the diastole, first in one side, then in the other. This in turn causes dilatation and finally hypertrophy*. Usually, enlargement of the right ventricle precedes that of the left, and complications may result from the accumulation of the blood in the chambers.

In our studies on peripheral arteriovenous fistula² and on the patent ductus arteriosus,³ a very intimate interrelationship between increased minute volume flow and cardiac enlargement was demonstrated. Additional corroborative information as to this interrelationship was obtained from a study of such developmental anomalies of the heart as accompany patent interventricular septum, patent foramen ovale, cor triloculare, and the associated abnormalities of pulmonary and aortic stenosis. In the presence of these intracardiac fistulas, the volume flow of blood through the heart is unequally distributed among the several chambers, and theoretically the developmental response in these chambers should be commensurate with the volume flow through them. Clinical evidence in favor of this view is provided by the following cases of congenital anomalies of the heart.

REPORT OF CASES

CASE 1 (Fig 1)⁴—A girl, aged 8 months, died from marasmus after being in the hospital for two days. The past history was one of malnutrition,

* From the Department of Surgery, Western Reserve University School of Medicine

* Presented before the Clinical Society of Surgery, Oct 18, 1924

1 Seham, Max. Congenital Heart Malformations, in Abt, I. A. Pediatrics, Philadelphia, W. B. Saunders Company, 1924

2 Holman, Emile. Experimental Studies in Arteriovenous Fistulas, Arch Surg 9 872-879 (Nov) 1924, Heart 9 337-341 (Dec) 1924

3 Holman, Emile. Certain Types of Congenital Heart Disease Interpreted as Intracardiac Arteriovenous and Veno-Arterial Fistulae, I, Patent Ducus Arteriosus, Bull Johns Hopkins Hosp 36 61-80 (Jan) 1925

4 Cautley. Two Specimens of Congenital Heart Disease, Proc Roy Soc Med, Section Dis of Child 2 34-36, 1908

anorexia, constipation and bottle feeding. The child was small and wasted, with an earthy complexion, a prominent sternum, beaded ribs, and hypertrophied heart without cardiac murmur, and an enlarged liver and spleen. There were no attacks of lividity or cyanosis.

At necropsy the heart was large and globular, weighing 4 ounces (113.4 gm). Superficially, it showed no indication of two ventricles, and the wall felt the same thickness throughout. The auricles were normal. The foramen ovale was closed. The auriculoventricular valves appeared normal and competent. The ventricular cavity was large and globular and devoid of a septum, but exhibited a thick convex arch of muscle which separated the aorta and the pulmonary artery and roughly divided off a small left ventricle, the infundibular portion, from the large, dilated right ventricle into which both auricles opened. The pulmonary artery was much dilated above its origin as far as the origin of the two main trunks. The ductus arteriosus was of small size, and could not be regarded as pervious. The dilatation of the pul-

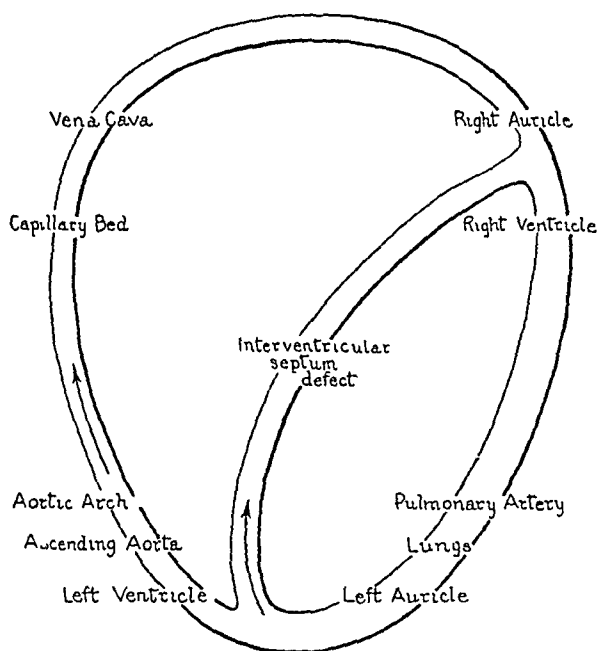


Fig 1—Patent septum acting as an arteriovenous fistula, no cyanosis, marked hypertrophy of right heart and dilatation of pulmonary artery, left ventricle and aorta small

monary artery was no doubt due to the large amount of blood driven into it at each systole. The aorta was small and received little blood. This accounted for the poor development of the child.

The patent septum in this case served as an arteriovenous fistula transmitting oxygenated blood from the left side of the heart to the right. There was undoubtedly a mingling of venous and arterial blood in the right ventricle, but the blood received by the left ventricle through a "normal auriculoventricular valve," though small in amount, was fully oxygenated, consequently, the systemic circulation received only arterial blood. This is confirmed by the clinical description of an earthy complexion and by the absence of cyanosis. The *cor triloculare*, without cyanosis, has been cited as evidence against the admixture theory of

cyanosis, but it is evident that such an interpretation is not valid. The difference in development between the small left ventricle, with its correspondingly small aorta, and the large right ventricle, with the dilated pulmonary artery, is attributed to the difference in volume flow of blood through them.

CASE 2 (Fig 2) ⁶—A boy, aged 15 months, had fairly well marked cyanosis. There was no clubbing of the fingers and toes. He suffered severe attacks of pain in the chest. The apex beat was forcible in the fifth space. There was cardiac dullness 1 inch (2.5 cm) to the right of the sternum and upward to the third costal cartilage. A rough systolic murmur was heard all over the precordia, loudest at the third costal cartilage and the third space close to the left border of the sternum, conducted upward toward the left shoulder.

At necropsy the right auricle was greatly distended, and the left very small. No patent foramen ovale was present. The right ventricle was dilated

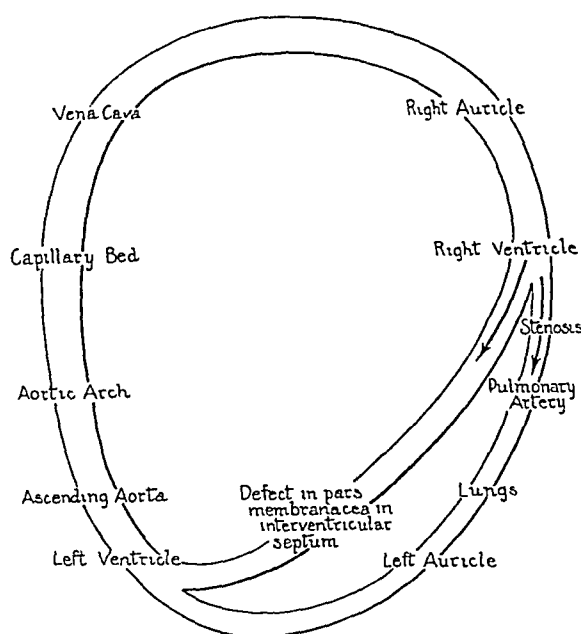


Fig 2—Septum defect acting as a veno-arterial fistula, cyanosis prominent, right auricle dilated, left auricle small, right ventricle dilated and hypertrophied, left ventricle also hypertrophied.

and its walls were greatly hypertrophied. There was stenosis of the pulmonary artery and of its orifice. The left ventricle was dilated and its walls were hypertrophied. There was an opening in the interventricular septum just below the aortic valves in the pars membranacea.

CASE 3 (Fig 2) ⁵—W. P., aged 20, had always been blue, but was considered to be fairly strong till puberty. At the age of 17, he had two slight attacks of rheumatism, he became of a deeper blue color and suffered from dyspnea. He was very cyanosed and had very bulbous fingers. A systolic murmur was audible all over the cardiac area, somewhat variable from time to

5 Sawyer, J. E. H. Congenital Malformations of the Heart, Pulmonary Stenosis and Patent Interventricular Septum, *Birmingham M. Rev.* 66:153-168, 1909.

6 Rolleston, H. D. Congenital Heart Disease, Pulmonary Stenosis, Patent Septum Ventriculorum, *Tr. Path. Soc. London* 43:32-33-34, 1892.

time, but loudest over the fourth interspace to the left of the sternum. No thrill was ever felt. Death occurred from hemoptysis and phthisis.

At necropsy the heart weighed 20 ounces (567 gm). The right auricle was both dilated and hypertrophied. The tricuspid valve was thickened. The right ventricle was hypertrophied so as to resemble the left ventricle, and was somewhat dilated. The pulmonary valves were welded together to form a funnel projecting up into the small thin walled pulmonary artery. The left auricle was normal and the fossa ovalis intact. The mitral valve was thickened and the left ventricle hypertrophied, but of about equal thickness with the right. The interventricular septum in the situation of the "undefended space" was patent. The circumference of this communication between the ventricles was $2\frac{7}{8}$ inches (7.1 cm), and its edges were rounded. The aortic orifice was directly over the septum, and that vessel may thus be said to have arisen from both ventricles. The aortic valves were somewhat thickened. The ductus arteriosus was obliterated and small.

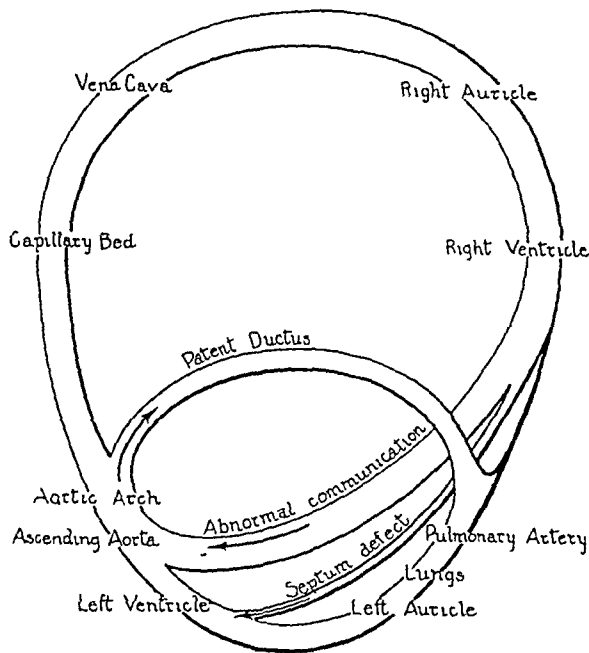


Fig 3—Abnormal opening between right ventricle and aorta acting as a veno-arterial fistula, patent ductus an arteriovenous fistula, cyanosis marked, right auricle dilated and hypertrophied, right ventricular wall thicker than left, pulmonary artery small, aorta very large

In Cases 2 and 3, the patent septum may be designated as a veno-arterial fistula transmitting unoxygenated blood from the right to the left sides of the heart, and in each instance cyanosis was a prominent feature. In each the right auricle was dilated and hypertrophied as compared to the left, which was "very small" or "normal." This difference is interpreted as the response to the stimulus of an increased flow through the right auricle as compared to the left. It was noted in each case that the two ventricles were hypertrophied and dilated, the right being equal to the left in size and development. The aortic orifice was noted in Case 3 as arising from both ventricles. Undoubtedly, the

right heart was responsible, equally with the left, in supplying the systemic circulation with blood and in overcoming the systemic peripheral resistance

CASE 4 (Fig 3)⁷—A girl, aged 11½ months, was first noted to have cyanosis at 6 months, this had been continuous more or less thereafter, with paroxysmal exacerbations. There was violet coloration of the hands and feet. No thrill was present. The pulse was 144. The respirations were tranquil. There was a systolic murmur and edema of the feet. Hemoglobin totaled 120, red blood cells, 6,417,000. There was dyspnea on the slightest exertion. The liver was enlarged. When the child cried there was intense cyanosis, this became more frequent and more intense in the last days of life. Death occurred in an attack of dyspnea and cyanosis.

At necropsy the heart was greatly enlarged, particularly on the right, which formed the apex. The heart musculature on the right was more firm than on the left. The musculature of the right auricle was hypertrophied, its cavity

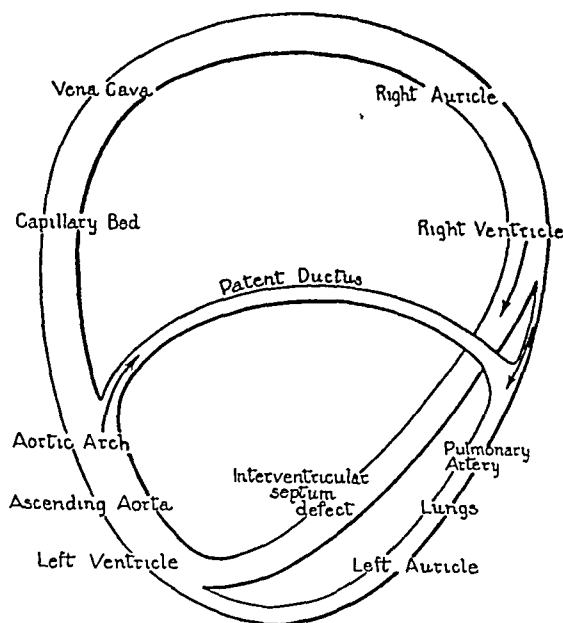


Fig 4—Septum defect acting as a veno-arterial fistula, patent ductus as an arteriovenous fistula, cyanosis prominent

dilated. The thickness of the right ventricle was from 7 to 8 mm, the thickness of the left ventricle, 6 mm. In the arch was the large opening of the ductus in communication with the pulmonary artery. The opening between the pulmonary artery and the right ventricle was completely obliterated. There was a free opening from the right ventricle into the aorta, this opening was much larger than that with the left ventricle. The opening between the ventricles was as large as a small pea. The aorta was very large—4 cm in circumference. The ductus was 6 by 3 mm. The pulmonary artery was much smaller than the aorta, and the wall was thinner.

CASE 5 (Fig 4)⁸—A boy, aged 9, was apparently in perfect health until 7 years old. About two years before this examination, he experienced dyspnea,

7 D'Espine and Mallet, H. Du Malformation Congenitale du Cœur avec Cyanose Paroxystique, *Rev de med* 28 941-949, 1908

8 Johnston, James. Cyanosis with Deficiency of the Interventricular Septum, *Brit M J* 2 351, 1872

pain across the chest and dizziness for the first time. He then had his first series of epileptic fits, and from that time on gradually became more cyanotic. The face and extremities were very blue. The lips were purple. The conjunctival membrane was congested. The apex beat was normal in position with a heaving rather than an accentuated impulse. There was a loud systolic murmur, harsh and rough in character, most distinct in the midsternum. This bruit was barely perceptible at the apex of the heart but was limited to a space on the sternum opposite the fourth left rib cartilage. There was no regurgitation in the veins. The bruit was most distinct in the horizontal position and frequently disappeared when the patient raised upright, but when this occurred he experienced a faintness and precordial pain, and then was convulsed. The lungs were fully distended. There was no increase in the number of respiratory movements, no cough, no expectoration, and he did not spit blood. The liver was not enlarged. The kidneys acted well. The first fit was noticed in the hospital about eight days after admission and fits then occurred at uncertain intervals until a week before his death. They were frequent during the last

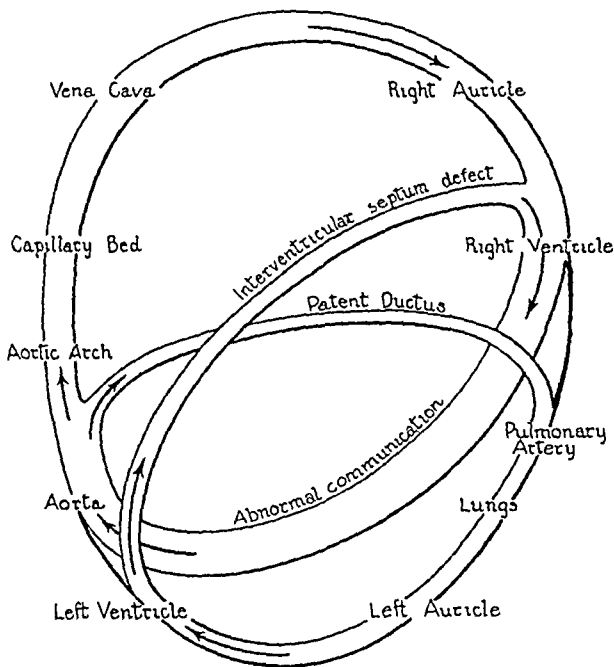


Fig 5—Transposition of aorta to arise from right ventricle, cyanosis marked from birth, right auricle greatly dilated, left auricle undeveloped, right ventricle much hypertrophied, left ventricle small, aorta large

week of life, with painful intermissions and deep cyanosis. About ten days before death symptoms of pulmonary congestion came on with cough and frothy expectoration, but no blood. Death occurred after a series of fits.

At necropsy every organ was found deeply congested with dark fluid blood. In the right auricle the inferior and superior cavae were normal in size and position. The foramen ovale was closed. The tricuspid orifice was perfect and the valves were healthy. In the right ventricle the pulmonary arterial orifice was so small that it hardly admitted an ordinary probe. Immediately above and to the right side of this orifice, the aorta could be felt opening into the ventricle. The septum ventriculorum was deficient, the finger could be pushed with ease into the aorta from either the right or the left ventricle. The aorta seemed to be placed over both ventricles so as to receive the blood from both as from a common cavity. The ductus arteriosus was patent in its normal position and the diameter of an ordinary goose quill, in length about one-eighth inch (0.3 cm). There can be no doubt that this state of the heart was congenital.

Cases 4 and 5 presented both a veno-arterial and an arteriovenous fistula in the same heart, the patent ductus in each case acting as the only pathway for the flow of blood through the lungs, and transmitting a mixture of arterial and venous blood from the aorta to the pulmonary artery. An abnormal communication transmitting such a mixture might properly be designated as a "veno-arteriovenous" fistula. In Case 4 the abnormal opening between the right ventricle and the aorta, and in Case 5 the septum defect acted as veno-arterial fistulas transmitting purely venous blood from the right heart into the systemic circulation, the volume of unaerated blood entering the aorta being greater than the volume of oxygenated blood from the left heart. This admixture of arterial and venous blood, with a preponderance of the latter, explains the high degree of cyanosis in each case. There is definite evidence, as will be discussed in a later article, that there occasionally occurs in the course of years a gradual narrowing and, finally, obliteration of the abnormal patent ductus widely open at birth, and it is to this fact that we attribute the ill health and cyanosis that followed seven years of normal good health in Case 5. As long as the patent ductus remained relatively large and transmitted a large amount of blood to the lungs for aeration, there was no cyanosis, and the oxygen interchange was sufficient for normal needs.

Case 4 also illustrates well the response of the heart chambers to the increased flow of blood through them, as evidenced by the dilatation and hypertrophy of the right auricle, and the greater thickness of the right ventricle as compared to the left. The right ventricle, in addition to its normal function, had, indeed, usurped part of the work of the left heart of propelling the stream of blood into and through the systemic circulation, and in consequence was proportionately more developed than normal.

CASE 6 (Fig 5) ⁹—A boy, aged 4, had been livid from birth and subject to frequent pulmonary attacks. He was small for his age and presented the signs of extreme cyanosis, with great clubbing of the ends of the fingers and toes. There was some bulging at the lower part of the sternum and to the right of the normal cardiac area, with a forcible diffused cardiac impulse, chiefly on the right side of the sternum. The apex beat was most plainly visible about three-fourths inch (20 mm) below the right nipple. No bruit could be heard at any time, but the first cardiac sound was doubled and extremely forcible, and the second sound everywhere accentuated. Air entered feebly at the extreme bases of the lungs. Death followed convulsions.

At necropsy the heart was larger than natural, the transverse diameter being almost as large as the vertical. The right auricle was enormously distended

9 Habershon, S. H. Congenital Malformation of Heart and Kidneys, Obliteration of Pulmonary Artery, Aorta Arising from the Right Ventricle, Imperfection of Septum Ventriculorum, Lungs Supplied from Aorta by a Ductus Arteriosus Dividing Into Right and Left Pulmonary Branches, Horse-Shoe Kidney, Tr. Path. Soc. London **39** 71-74, 1887-1888.

and filled with black fluid blood. In situ only one vessel (the aorta) could be seen arising from a central position. When the cavities of the heart were opened, the right auricle was found to be dilated and thin walled, the vessels entered it normally, and the foramen ovale was closed. The right auriculo-ventricular valves were healthy and competent, but imperfectly divided into two unequal sized curtains. The right ventricle was enlarged and its walls were much hypertrophied. The aorta arose directly from the right ventricle, and was of unusually large size. The sigmoid valves were very large and loose. Immediately below their attachment was an imperfection in the base of the septum of a size sufficient to admit the little finger. The aorta must, therefore, have received blood directly from both ventricles during life. The left ventricle was of small size, its walls hypertrophied, but not to the same extent as the right. There was a normal appearing opening into it from the left auricle through a bicuspid valve, and a communication through the septum with the cavity of the right ventricle and with the aorta. No vessel opened from the left ventricle, and there was no trace of a pulmonary artery from

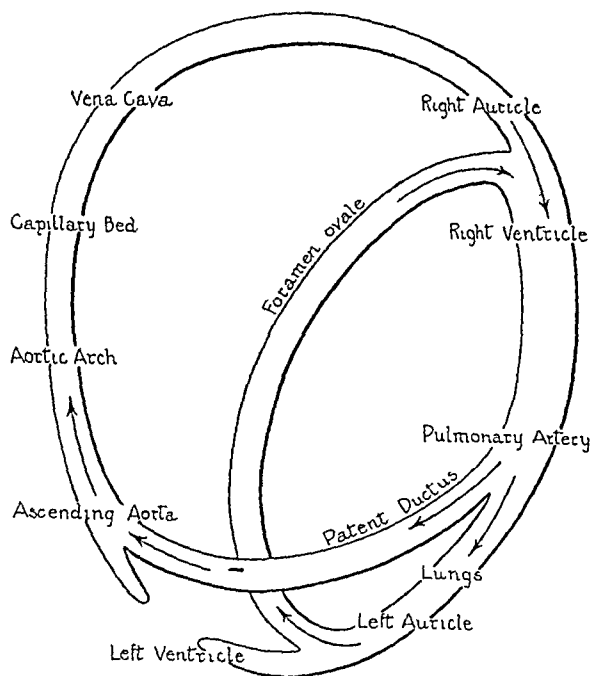


Fig 6—Right ventricle dilated and hypertrophied, left ventricle greatly reduced, pulmonary artery 29 mm in diameter at its origin, 14 mm in diameter after giving off patent ductus

within the ventricle. The left auricle was atrophied and reduced to a venous sinus, into which the pulmonary veins opened. The left appendix auricle was present, but was very small. The aorta was of unusually large size. Immediately beyond the point where the great vessels were given off it became suddenly constricted to about half its previous dimension. The lungs were supplied from the aorta by means of a short trunk about one-fourth inch (0.6 cm) in diameter, which was evidently the ductus arteriosus, and divided into a right and left pulmonary artery. Joining the right pulmonary branch was a vessel which could be traced to the heart to a point on the left of and a little anterior to the aorta. It was there lost in the muscular wall of the right ventricle. The lungs were congested but there was no collapse.

The lividity from birth in this patient is readily explained by the direct transfer of venous blood from the right heart into the systemic circulation. Only a minor portion of the circulating blood passed

through the patent ductus for aeration in the lungs. The differences in size between the enormously dilated right auricle and the atrophied left auricle, between the small left ventricle and the large hypertrophied right ventricle, are dependent on the unequal volumes of blood flowing through them.

CASE 7 (Fig 6)¹⁰—A boy, aged 3 weeks, had dyspnea. Death occurred by suffocation.

At necropsy the right half of the heart appeared to be greatly hypertrophied. The right ventricle was greatly dilated. The pulmonary artery above the valves measured 29 mm across, after the origin of the ductus botalli, 14 mm. The foramen ovale was open and broad. The circumference of the mitral opening measured about 22 mm. Below the mitral was found the greatly reduced left ventricle. The pulmonary artery communicated with the aorta by a 15 mm

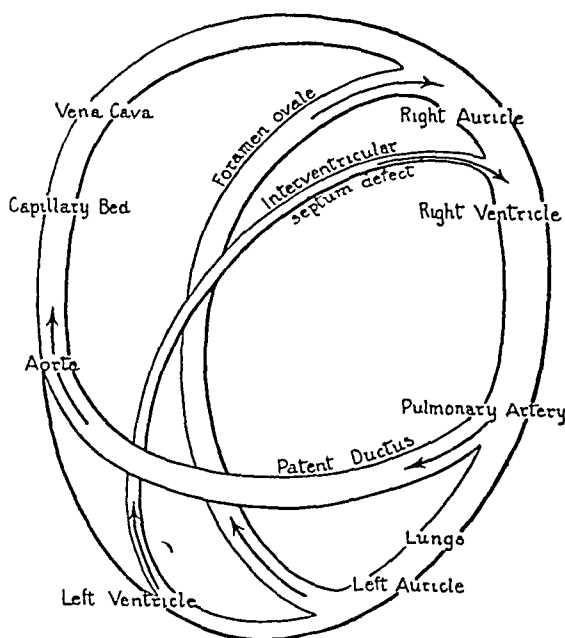


Fig 7—Foramen ovale and patent septum acting as arteriovenous fistulas, the patent ductus as a veno-arterial fistula, right auricle larger than left, right ventricular wall three times thicker than left. The patient had had cyanosis from birth.

long ductus botalli. The aorta measured, after the opening of the ductus botalli, 15 mm, on the cardiac side of this opening, 10.5 mm, and before giving off the vessels of the head and neck, 7.5 mm. No communication between the aorta and the heart cavities was found. The heart weighed 41 gm.

Case 7 illustrates in a striking manner the interrelationship between the size of the large vessels and the volume flow of blood through them. The diameter of the pulmonary artery was exactly halved after giving off the patent ductus, and the aorta was considerably enlarged beyond the entrance of the ductus.

¹⁰ Martens, Gustav. Zwei Fälle von Aorten Atresie, Virchows Arch^h f path anat 121 322-335, 1890.

CASE 8 (Fig 7)¹¹—An infant, 6 months old, was small and underdeveloped. The pulse was 130. The child was dyspneic and very cyanotic from birth. The heart sounds were clear, no thrill was heard.

At necropsy the heart was one and one-half times as large as the fist. The left auricle was smaller than the right. There was no aorta from the left ventricle. The right ventricle formed the apex of the heart. The musculature of the right was thicker than the left. The pulmonary artery was 1.4 cm broad. The usual large arteries came from this pulmonary artery. The ductus was 6 mm in diameter. There were two branches to the lungs. The foramen ovale measured 5 by 3 mm. The left ventricular wall was from 3 to 5 mm thick. The right ventricular wall was from 0.8 to 1.2 cm thick. There was a "pfennig stuck groszes" round opening between the two ventricles. Cyanotic induration of organs was present.

CASE 9 (Fig 8)¹²—A boy, aged 5 months, was a patient from the age of 3 weeks. The child was cyanosed, sometimes deeply, sometimes only slightly. It had no clubbing of the fingers or toes. It was always very short of breath, its respirations being usually about 60 per minute. Its pulse varied from 160

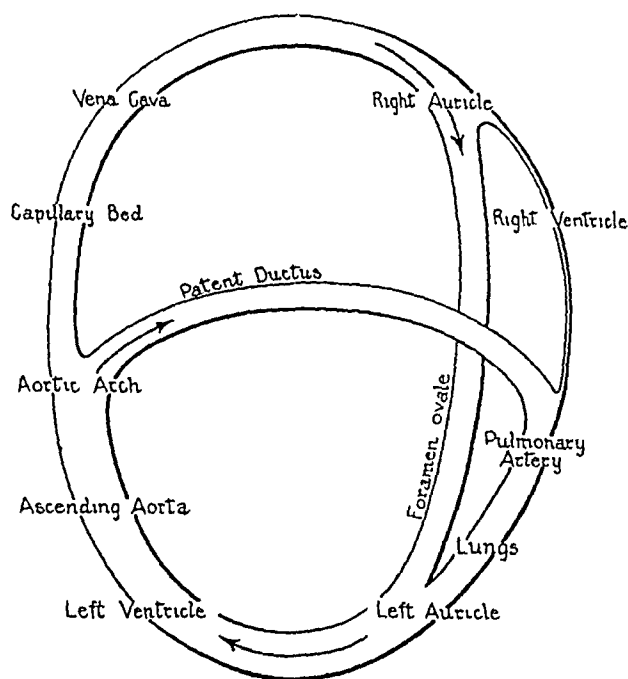


Fig 8—Foramen ovale acting as a veno-arterial fistula, the patent ductus as an arteriovenous fistula, cyanosis present, right auricle dilated and hypertrophied, right ventricle undeveloped, left ventricle abnormally dilated and hypertrophied.

to 180. It never sucked very well. The first and second heart sounds were audible. There was no murmur at any time. The general character of the sounds approached that of the sounds of the fetal heart. The ultimate cause of death was pulmonary catarrh.

At necropsy the aortic arch was somewhat disproportionately large, and arose from an abnormally large and thick left ventricle. A mitral valve with a partial third flap opened into the left ventricle from a left auricle of about normal size. The mitral flap bore the normal relation to the aortic valves,

11 Bokay, Z. Ein Fall von Persistierendem Truncus Arteriosus Communis bei einem 6 Monate Alten Säugling, *Jahr f Kinderh*, Berlin 80 327-334, 1914.

12 Moore, Norman. Congenital Malformation of Heart, *Tr Path Soc London* 43:31-32, 1892.

which were also normal. The foramen ovale was open, and the right auricle was of double the normal capacity and thickness. There was no true tricuspid valve, but only an opening the size of a common pin, which led into a right ventricle not larger than two pin heads. This had no communication with the left ventricle. A minute pulmonary artery without valves issued from the right ventricle. This pulmonary artery grew much wider as it approached the ductus arteriosus, which was patent and nearly as wide as the pulmonary artery.

The circulation was chiefly, if not entirely, carried on by the single ventricle, the pulmonary blood stream being directed through the ductus arteriosus from the aorta. The blood from the hypertrophied right auricle was driven into the left auricle, and thence, mixed with the aerated blood from the lungs, went through the wide mitral orifice into the left ventricle. There was no unmixed blood current. The arteries from the arch of the aorta were normal. The other viscera of the chest and abdomen showed no abnormality.

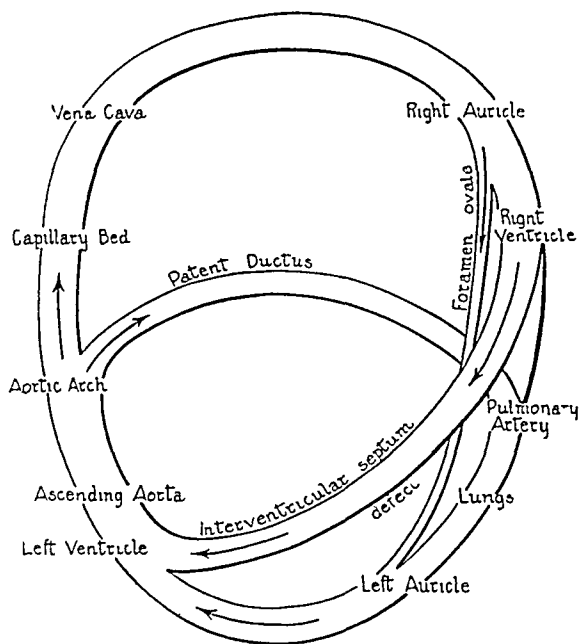


Fig 9—Septum defect acting as a veno-arterial fistula, the patent ductus as an arteriovenous fistula, intermittent cyanosis, right auricle and right ventricle dilated and hypertrophied, aorta very wide at origin, greatly diminished after giving off patent ductus

CASE 10 (Fig 9)¹³—A girl, aged 9 months, was small with an ash gray tint. She was cyanosed during coughing or crying. A loud systolic murmur was heard, and there was increasing dyspnea and weakness.

At necropsy the right auricle and right ventricle were both of abnormally large capacity, their walls hypertrophied. The foramen ovale was patent. The septum of the ventricles was deficient at its upper part, the aortic orifice being so placed as to communicate as directly with the right as with the left ventricle. There was no opening into the pulmonary artery. The aorta was very wide in the ascending portion of its arch, then tapered till it was joined by the ductus arteriosus, when it suddenly became much smaller. The ductus arteriosus was rather wide and its coats were thick, and the right and left pulmonary arteries, both from their direction and their structure, appeared to be branches of the arterial duct coming from the aorta, rather than of the impervious vessel coming from the heart. The lungs presented congestion of their posterior parts and extensive collapse of the lower lobes.

CASE 11 (Fig 10)¹⁴—A man, aged 31, had been noticed to have a livid appearance from his birth, but he did not appear to have had any symptoms directly referable to the heart until he reached his twenty-seventh year. In November, 1878, he had some swelling of the abdomen, became short of breath, and suffered from slight hemoptysis. July 3, 1882, he was admitted into the Middlesex Hospital suffering with pain in the abdomen and loins. The vessels of the face were markedly injected, the lips were livid, and the finger nails of a slaty bluish color. There was a slight presystolic thrill. The cardiac dulness commenced above at the upper border of the fourth rib, extended to the nipple on the left, and to the border of the sternum on the right. On auscultation the first sound at the apex was very rough, the second sound sharp and ringing. At the base, in the third left interspace close to the sternum, a loud rasping bruit could be heard. This bruit, which was very localized, appeared to follow closely on and partly to conceal the second sound. It was not audible at the aortic cartilage nor at the back. The pulse, which was 96, was of fair volume, compressible and regular. The patient died.

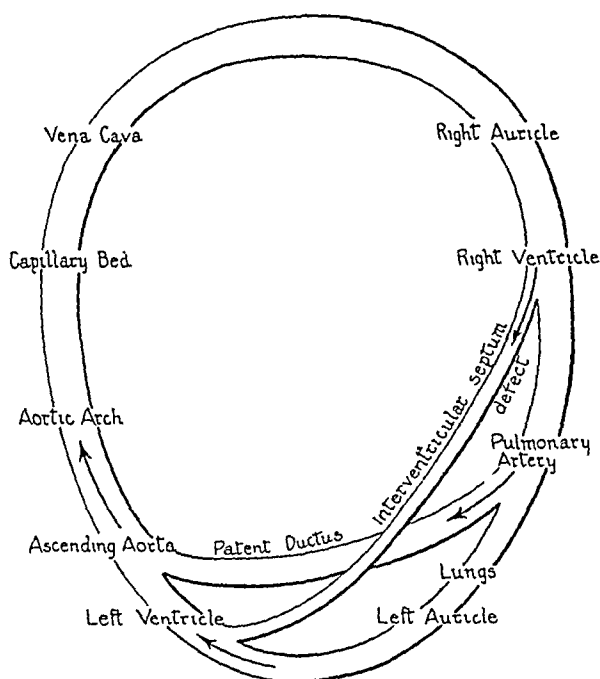


Fig 10—Patent septum and patent ductus acting as veno-arterial fistulas, right ventricular wall thicker than left. Cyanosis had been prominent from birth.

At necropsy the peritoneal cavity contained about one-half pint (236.5 cc) of serum. The pericardial sac contained 30 ounces (887.2 cc) of serum, and the serous surfaces, especially at the base of the heart, were coated with shreds of lymph. The heart weighed 26 ounces (737 gm), all the cavities were hypertrophied, and the auricles also notably dilated. Coagula occurred in all the chambers. The tricuspid orifice was large, and its valves incompetent, the pulmonary valves were natural and competent, and the infundibulum was natural. The ductus arteriosus, which had been partially laid open in removing the heart, was of large size and freely patent, admitting the tip of the little finger. At its orifice into the aorta there was some calcified atheroma beneath the lining membrane. The aortic valves were competent, the mitral valve

¹⁴ Coupland, Sidney. Congenital Malformation of the Heart, Patent Ductus Arteriosus, Defect of Ventricular Septum, Cyanosis, Facial Erysipelas, Pericarditis—Death, *M. Times & Gaz.*, London 2 501, 1884.

also was competent, but thickened along its border. A rounded aperture large enough to permit the passage of an ordinary cedar pencil occurred in the pars membranacea of the ventricular septum. Whether or not the aorta was narrowed beyond the entrance of the ductus was not observed. The wall of the right ventricle at the base of the heart measured seven-eighths inch (2.1 cm) in thickness, that of the left six-eighths inch (1.8 cm). The lungs were highly edematous.

COMMENT

In surveying these remarkable diversions from the normal flow of blood, most instructive information is obtained as to the occurrence and causation of cyanosis. It is evident that cyanosis in all instances was due to the flow of blood through abnormal openings from the venous to the arterial sides of the circulation. These abnormal openings may, therefore, be designated as *veno-arterial* fistulas¹⁵. In those instances in which the admixture occurred in the opposite direction, i. e., from the arterial to the venous side of the circulation, as in Case 1 and in numerous cases of patent ductus, no cyanosis was recorded. Under such circumstances the abnormal opening serves as an arteriovenous fistula, transmitting only aerated blood. The most intense cyanosis was encountered in Cases 6, 8 and 10, in which the greater part of the blood from the vena cava entirely escaped the lungs and was diverted, unoxygenated, through abnormal openings into the systemic circulation. That cyanosis may be due to the admixture of venous to arterial blood is abundantly proved by these examples, as well as by those cases of pure patent ductus in which the blood flows from the pulmonary artery into the aorta. It is apparent that the authors of books on pediatrics should revise their statements that such an admixture does not explain the cyanosis found in cardiac anomalies¹⁶.

Abundant evidence is also provided by these circulatory anomalies to substantiate the physiologic principle that the chambers of the heart, as well as the blood vessels, place themselves in harmony with the volume

15 Since the completion of this article and a preceding one on patent ductus arteriosus, my attention has been called to a contribution on the subject by Dr. Maude Abbott, with W. T. Dawson. *The Clinical Classification of Congenital Cardiac Disease*, Internat. Clin. 4, Series 34, December, 1924. Dr. Abbott designates the abnormal openings delivering venous blood into the systemic circulation as "venous arterial shunts," and those delivering the arterial blood into the pulmonary circuit as "arterial venous shunts." Although she does not include the patent ductus as a "venous arterial" shunt, it would seem from our studies that it should also be included under this head. Cyanosis in these cases depends, of course, on the amount of unaerated blood that escapes into the systemic circulation, and obviously does not appear unless the diversion of venous blood has assumed considerable proportions.

16 Griffith, J. P. C. *The Diseases of Infants and Children*, Philadelphia, W. B. Saunders Company, 1921, p. 126. Graham, E. E. *Diseases of Children*, Philadelphia, Lea and Febiger, 1916. Tuttle and Hurford. *Diseases of Children*, Philadelphia, Lea and Febiger, 1917. Holt and Howland. *Diseases of Infancy and Childhood*, New York, D. Appleton & Co., 1922.

flow of blood through them. The enlargement of the heart that is found in these cases, restricted to certain chambers only, may be both a dilatation and an hypertrophy, the dilatation in the well functioning organ being solely attributable to the increased minute volume flow through those chambers. The hypertrophy, however, may be attributed to two factors: (1) the increased effort necessary to propel forward an increased minute volume flow of blood, and (2) the assumption by the right heart, in addition to its normal function, of a part of the burden of the left heart in overcoming the systemic peripheral resistance and in supplying the systemic circulation with blood. Whenever abnormal openings, such as the septum defect or the patent ductus, place the right heart in direct communication with the systemic circulation, an increased development of the right ventricle is observed. When, as in Case 9 (Fig 8), the left heart assumes the full burden of supplying both the systemic and the pulmonary circulations, we have a proportionately greater development of this half of the heart.

The clinical evidence presented by this study of congenital cardiac anomalies is convincing that the heart chambers respond, both by dilatation and by hypertrophy, to an increased volume flow of blood through them, and the deduction is justified that such an increased flow is the most effective physiologic stimulus to cardiac dilatation and hypertrophy.

THE USE OF UREA AS A DIURETIC IN ADVANCED HEART FAILURE *

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It has been known for a long time that the administration of urea in large doses produces a considerable increase in the volume of urine. Urea was, however, until comparatively recently little utilized for its diuretic properties. This was doubtless due to the prevailing idea that urea retention was the causative factor in uremia. Urea was considered a harmful substance and any measures undertaken to raise its concentration in the body were regarded with disfavor. With the advance of clinical chemistry it soon came to be recognized that although the urea in the blood is raised in uremia it is not the cause of the condition. It became established that if the function of the kidney is good urea can be given to patients for prolonged periods of time with impunity. Even in cases in which kidney disease was known to be present, large doses of urea have been given in order to estimate the functional capacity of the kidney and no untoward results have been observed. Recently, urea has been administered over prolonged periods of time in the treatment of certain cases of nephritic edema in which the blood urea was not raised and very favorable results have been reported.

Urea was first used as a diuretic by Friedrich¹ in 1892. In this investigation he administered urea in edema due to various causes, the series included a few cases of cardiac edema. He gave from 2 to 14 gm a day and reported very favorable results. Feilchenfeld² conducted a similar study with similar results. He stated that the increase in urine volume was proportional to the dose. Strauss³ gave urea in doses up to 100 gm a day to twelve patients with cardiac edema following heart disease or arteriosclerosis, with very beneficial results. He commenced with doses of 40 gm a day and gradually increased it as necessity demanded. In spite of the favorable results reported by the foregoing observers, urea has not come into common use as a diuretic in cardiac edema for reasons we have not been able to ascertain.

* From the Hospital of the Rockefeller Institute for Medical Research

1 Friedrich, W. (Ueber die harntreibende Eigenschaft des Uream) as ureum hugyhajto tulajdonsargarol Kozlemny u budapeste Magy kir tud Egyetem gyogyozertani intezelebol, Magy orv Arch **1** 400-415, 1892

2 Feilchenfeld, J. Ueber Harnstoff als Diureticum, Therap d Gegenw **59** 273, 1918

3 Strauss, H. Ueber Harnstoff als Diuretikum, **58** 375, 1921

For our investigation we have selected cases of advanced cardiac decompensation which have responded only partially or not at all to the methods usually employed in the treatment of cardiac edema. The series included four cases of mitral stenosis with auricular fibrillation, two cases of mitral stenosis, one case of aortic disease with auricular fibrillation, and one case of exophthalmic goiter with auricular fibrillation.

The routine treatment of all patients included rest in bed, a salt free diet, thorough digitalization, and restriction of the fluid intake to 1,200 c c a day. Four of the patients had also received novasurol, and had been rendered edema free, but after the injections of this drug had been discontinued, the urine volume was subnormal and edema had gradually reappeared. Urea was given with a view to reestablishing a normal water balance by maintaining an adequate urine output, and also, when slight edema had collected, to bring about its removal. The other four patients of our series all exhibited marked edema when urea medication was instituted. These patients had not received a course of novasurol. One case of mitral stenosis with regular rhythm (Case 6) did not receive digitalis as we decided to study the effect of urea without previous digitalization.

In all cases twelve hour specimens of urine were collected, and in these, the volume, the specific gravity and the chlorid excretion were determined. Urea administration was not commenced until the urine volume and chlorid excretion had reached a constant level. In order to study in detail the changes that took place in the urine volume, urea excretion and blood urea, and their time relations, we collected a specimen of urine over a period of seventy-two minutes at frequent intervals and obtained a specimen of blood in the middle of this period. The time of making these tests was varied with relation to the administration of urea in order that its effect might be followed more closely. During these periods no food or fluid was given. The urea in the blood and urine was estimated by the method of Van Slyke and Cullen,⁴ the chlorid in the urine by a Volhard titration, and the chlorid in the plasma by the method of Van Slyke.⁵

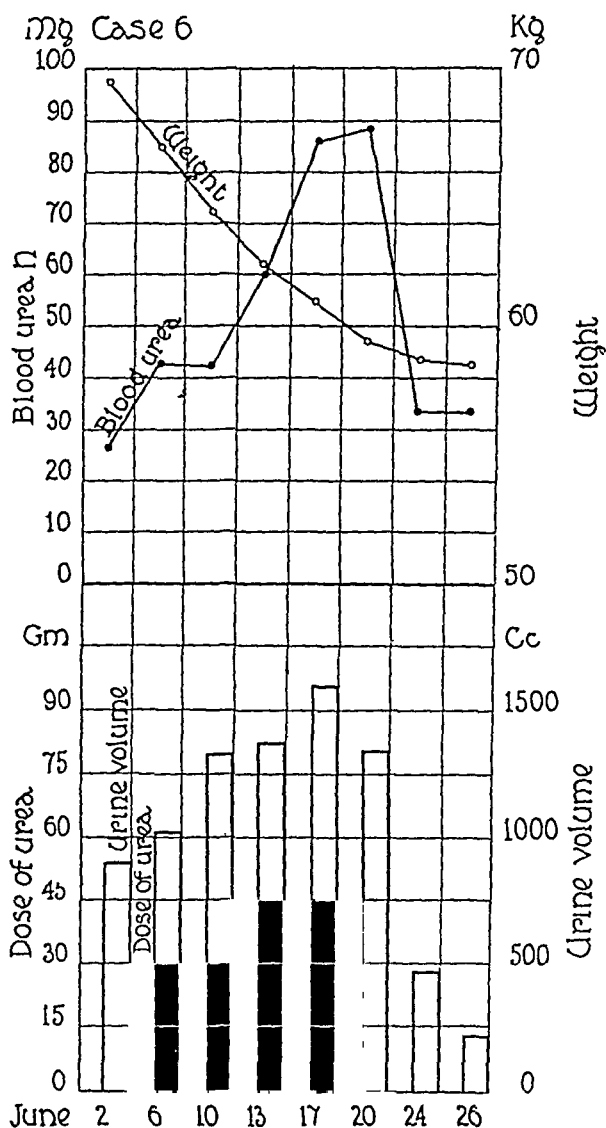
METHOD OF ADMINISTRATION

Urea was given in a small quantity of water a short time after a meal as it had been previously found that gastric disturbance was less likely to take place if it were given at this time. Urea has a peculiar metallic

4 Van Slyke, D. D., and Cullen, G. E. A Permanent Preparation of Urease and Its Use in the Determination of Urea, *J. Biol. Chem.* **19** 211, 1914.

5 Van Slyke, D. D. The Determination of Chlorids in Blood and Tissues, *J. Biol. Chem.* **58** 523 (Dec.) 1923.

taste and is not pleasant to take. The patients soon become accustomed to it, however. In four of our cases we administered 30 gm a day in divided doses of 15 gm. One dose was given at 8 30 a m and the other at 4 p m. In the other four cases, we studied the effect of increasing the quantity of urea. At first 30 gm a day was given. This



Effect of urea administration on urine volume, weight and blood urea. The patient had received one-third the daily dose of urea at 8 30 and 12 30, specimens of blood were obtained at 2 p m.

was then increased to 45 gm a day, and in two cases it was further increased to 60 gm a day. In three of the cases these increases took place at weekly intervals. The urea was given in three equal doses at 8 30 a m, 12 30 and 6 p m.

OBSERVATIONS

Urine Volume—The response of the cases with edema to urea feeding was prompt and, as a rule, very efficient (accompanying chart) In seven cases of the series, oliguria was present before treatment was instituted Their average daily output during the control period varied from 340 to 776 c c After 30 gm of urea a day, the average daily urine volume for each case varied, as a rule, between 900 and 1,300 c c When the amount was increased to 45 gm a day, the average daily output increased to 1,400 and 1,500 c c A dose of 60 gm daily was followed by a further slight increase (Tables 1, 2 and 3) The maximum response to a particular dose did not occur until the second or even the third day that the urea was given The daily output then remained at this level as long as this particular dosage was continued After urea administration was discontinued, a diuretic action was still distinguishable during the first twenty-four hour period thereafter

The time relations between the dosage and the diuretic response can be still further analyzed by studying the rate of excretion during the seventy-two minute periods From these data it may be seen (Table 2) that an increased urine volume was already established within three hours after the first dose was given After the urea feeding had been discontinued, the duration of its diuretic action depended to some extent on the dosage When 30 gm a day was given, its effect had practically passed off within fifteen to seventeen hours But with larger doses, its influence was still apparent at this time (Table 1)

Weight of Patient—Patients with edema lost weight corresponding to the diuresis that took place This loss of weight was gradual and continuous (chart) Patient 7, who did not have oliguria during the control period, is particularly noteworthy In this case the diuresis was much greater than in any of the others and the weight loss much more rapid, amounting to 8 kg in the first five days, this is to be contrasted with a loss of 1.6 kg in the preceding control period of five days In some cases continuance of urea feeding was necessary to prevent accumulation of edema, for when it was stopped the patient's weight tended to increase, until visible edema reappeared (Tables 1, 2 and 3)

Clinical Condition—Corresponding to the weight loss, one noted a disappearance of edema, and of transudates from the body cavities It was possible to clear all the patients of excess fluid in this way, with the exception of Patient 8 At the same time definite subjective improvement occurred The most striking clinical improvement occurred in Case 7, in which rapid subjective improvement occurred simultaneously with the loss of edema Patients 4, 5 and 6 also experienced very definite symptomatic relief which other measures had failed to give

This relief was associated with a decrease in weight. Cases 1 and 2 were free from edema at the beginning of treatment, and there was no definite symptomatic improvement due to urea. Case 3 improved subjectively when urea medication was commenced, although edema was absent and weight loss did not occur. The value of urea in the last three cases was in maintaining a normal water balance. When the urine volume diminished consequent on the withdrawal of urea, edema tended to recur, with its concomitant symptoms. When the administration of urea was resumed, symptoms and edema were again controlled.

In Case 8, urea administration did not result in clinical improvement. This was a case of mitral stenosis with normal rhythm in which there was considerable ascites, and slight edema of the legs. The patient reacted to a slight extent to digitalis, but this effect was transitory. The dose of urea was gradually increased up to 60 gm a day, each increase causing a definite rise in urine volume. Nevertheless, the clinical condition did not improve. After nineteen days of treatment the patient had lost 4.5 kg in weight. The patient began to complain of nausea and on the last day of the course vomiting occurred. The blood urea nitrogen was high after the midday dose, 74.9 mg per hundred cubic centimeters. Accordingly, urea was discontinued. The urine volume immediately fell and the general state of the patient became worse. It then became apparent that urea had been beneficial to the extent of postponing the unfavorable clinical course that subsequently developed.

Evidence of Intolerance—When the dose was taken on an empty stomach, vomiting occasionally occurred immediately after, but this symptom gave little trouble when the urea was given after meals. In three of the cases (Cases 5, 7 and 8) the prolonged administration of urea in large doses gave rise to untoward symptoms. These manifestations consisted in loss of appetite, nausea and sometimes vomiting, and in one instance, a feeling of weakness and lassitude. In Cases 7 and 8 they were sufficiently urgent to call for the discontinuance of urea administration. In these cases the blood urea nitrogen, estimated in the afternoon after the second daily dose, was 89.0 mg and 74.9 mg per hundred cubic centimeters, respectively. These figures are practically identical with those found by Hewlett, Gilbert and Wickett⁶ in experiments designed to investigate the toxicity of urea. In normal subjects they found that similar symptoms occurred when the blood urea reached values above 150 mg per hundred cubic centimeters (equivalent to 70 mg of urea nitrogen per hundred cubic centimeters). In our cases the symptoms disappeared, as a rule, within twenty-four hours after urea

⁶ Hewlett, A. W., Gilbert, Q. O., and Wickett, A. D. The Toxic Effects of Urea on Normal Individuals, *Arch. Int. Med.* **18**: 636 (Nov.) 1916.

TABLE 1—Effect of Urea on Urine Volume and Body Weight, the Blood Urea Nitrogen from Fifteen to Seventeen Hours After Urea Was Given, with the Rate of Urea and Urine Excretion at This Time

Case	Disease	Age	Dose of Urea, Gm	Number of Days on Which Urea Was Given	Weight at End of Period, Kg	Average Daily Urine Volume, Cc	Chlorid Excretion in 24 Hours as Sodium Chlorid, Gm	Blood Urea Nitrogen per 100 Cc Mg	Rate of Urine Excretion per 24 Hours Calculated from 72 Minute Period, Cc	Urea Nitrogen Excretion Rate per 24 Hours Calculated from 72 Minute Period, Gm	Urea Excretion Index 10 D	Remarks
1	Aortic disease (syphilitic) auricular fibrillation	47	30	7	90.6	318	1.63	13.1	700	4.64	74.2	Before urea
			30	4	87.6	1,069	2.47					
			30	8	84.3	1,280	1.89	14.7	560	5.70	56.0	Novasurol, 1 cc on second day*
			45	5	86.0	1,544	0.77	25.8	1,460	18.20	63.2	
			45	10	85.2	1,370	0.86	35.8	1,680	24.32	36.7	
			60	14	85.5	875	1.65					
			60	14	85.5	1,538	0.62	37.6	1,520	23.70	57.3	
2	Mitral stenosis, auricular fibrillation	31	30	6	73.4	585	2.70	10.5	340	1.64	36.6	Before urea
			30	4	73.8	980	3.09	15.8	720	9.34	95.2	
			30	3	74.0	931	2.15					
			30	11	74.4	743	3.96	10.6	888	5.62	76.9	
			30	11	73.9	985	3.15	18.8	696	9.68	84.3	
3	Mitral stenosis, auricular fibrillation	37	30	27	70.8	385	0.68	11.4	656	1.97	29.9	Before urea
			30	4	71.4	1,012	0.27	22.7	618	11.10	85.1	
			30	4	52.5	914	0.21	25.5	700	10.60	66.8	
4	Mitral stenosis, auricular fibrillation	20	30	6	41.1	447	1.70	14.3	460	1.10	69.2	Before urea
			30	12	38.2	863	2.37	24.7	780	4.64	34.8	Novasurol, 2 cc on fifth day*
			30	4	38.3	881	2.82	28.1	744	9.05	71.5	
			30	4	38.0	773	0.90	10.6	780	9.64	94.0	
			30	11	38.3	708	2.24	8.2	700	4.57	109.1	Novasurol, 2 cc on fourth day*
			30	11	37.2	845	2.48					Before urea
5	Exophthalmic goiter, auricular fibrillation, ascites, slight edema of the legs	46	30	4	44.9	688	2.86	15.7	1,000	4.72	47.0	Before urea
			30	9	44.0	1,423	3.67	19.4	900	8.44	70.9	
			30	6	41.8	1,199	2.69					
			30	5	43.1	640	1.28	24.3	860	5.19	35.6	
			30	5	42.5	1,413	3.42					
			30	5	42.1	1,298	3.47	6.6	560	2.31	31.5	
			30	5	42.1	664	2.12					
			30	22	44.1	910	1.81					
			15	15	42.7	1,339	3.89					
			15	15	42.2	1,556	1.70	36.6	560	6.62	37.3	
			15	5	42.0	1,125	0.51					

* Days on which novasurol was effective are not included.

was discontinued. In Case 6, however, they persisted to a lessened extent for four days thereafter. This case had a coexistent nephritis. After urea had been discontinued on account of symptoms of intolerance, it was always possible after a short interval to resume treatment, with a smaller dose, without the production of untoward symptoms.

Most of the patients, although not all, complained of thirst while under urea medication, however, this was never severe enough to interfere with the continuation of treatment. Headache was an occasional but never a severe cause of complaint. It is not certain that any of these symptoms were entirely due to urea administration, the benefits derived from the urea were marked and in our judgment far outweighed the discomfort entailed.

CHANGES IN URINARY CONSTITUENTS

Urea—A study of the rate of urea excretion shows that this ran parallel with the water excretion. The amount gradually increased for two or three days after treatment was begun and thereafter remained practically constant as long as the dosage remained constant, irrespective of the time it was continued. With larger doses the amount of urea excreted was increased. Cessation of treatment rapidly reduced the output of urea to its previous level. The increased excretion of urea began within from one and one-half to three hours after its administration (Table 2). Later in the day, after a second dose of urea had been given, the amount excreted was still greater (Table 3). On the following morning much less urea was excreted, the amount depending largely on the dosage. With the smaller doses, the quantity of urea in the urine seventeen hours after the last dose was only raised to a slight extent over the amount excreted before urea was given. With the larger doses, however, an increased output was still maintained at this time (Table 1).

Chlorid—As a rule more chlorid is excreted during urea administration than during the control period. The changes in amount are slight, however, and show very little regularity.

Abnormal Urinary Changes—We have seen no evidence of kidney irritation as shown by the presence of albumin, red blood cells or casts. When these were present before treatment was begun they became progressively reduced in amount and in almost every case disappeared.

CHANGES IN THE BLOOD

Urea—The blood urea nitrogen was increased by taking urea. The extent of the increase varied with the dose (chart). When a certain amount of urea was given on succeeding days in divided doses, the hours of administration being the same on these days, the concentration

TABLE 2—Effect of Urea on Urine Volume and Body Weight, the Blood Urea Nitrogen Shortly After the Morning Dose (One and a Half to Three Hours) During Time Urea Was Given Twice Daily, with the Rate of Urea and Urine Excretion at This Time

Case	Disease	Age	Dose of Urea, Gm	Number of Days on Which Urea Was Given	Weight at End of Period, Kg	Average Daily Urine Volume, Cc	Chlorid Excretion in 24 Hours as Sodium Chlorid, Gm	Blood Urea Nitrogen per 100 Cc, Mg	Rate of Urine Excretion per 24 Hours Calculated from 72 Minute Period, Cc	Urea Nitrogen Excretion Rate per 24 Hours Calculated from 72 Minute Period, Gm	Urea Excretion Index,		Remarks
											10 D	B \sqrt{VW}	
2	Mitral stenosis, auricular fibrillation	31	30	32	53.4	585	2.70	10.5	340	1.64	36.6	36.6	Before urea
					54.9	837	2.73	24.4	1,336	23.50	114.0	114.0	Received 15 gm of
					54.6	461	1.15	10.4	576	5.24	90.8	90.8	urea 1½ hours before bleeding
			30	10	55.4	1,017	2.21	25.5	1,392	23.45	106.6	106.6	
3	Mitral stenosis, auricular fibrillation	37	30	2	50.8	385	0.08	11.4	656	1.97	29.9	29.9	Before urea
					51.0	940	0.50	25.1	1,056	16.47	89.5	89.5	Received 15 gm of
					52.3	1,043	0.17	36.8	864	16.94	69.4	69.4	urea 3 hours before bleeding

of urea in the blood *at a given time* rose for a few days, until a certain height was reached. It continued at about this level however long this dosage was continued. As soon as the administration of urea was discontinued, the amount of urea nitrogen in the blood rapidly fell to its previous level. The volume of urine and urea excretion reflected the concentration of the urea in the blood. Seventeen hours after the last dose the urea in the blood was still raised—the height varying with the amount administered—but the increase was not great (Table 1). The urea given must have been rapidly absorbed for the amount of urea nitrogen in the blood increased within three hours (Table 2).

Plasma Chlorid—The chlorid in the plasma remained practically unaltered.

INDEX OF UREA EXCRETION

A study of kidney function by the urea excretion index of Austin, Stillman and Van Slyke⁷ is of considerable interest. These authors have made a study of the changes in the rate of urea excretion with varying concentrations of urea in the blood and with varying urine volumes. They have expressed the relationships between these factors in the following equation $K = \frac{10 D}{B \sqrt{VW}}$, in which D represents urea output (as grams per twenty-four hours), B the blood urea (as grams per liter), V the volume output (as liters per twenty-four hours), W the body weight (as kilograms), and K the excretory constant. For the normal subject K varies between 45 and 105. In kidney disease, values varying between 3 and the normal values have been observed.

With the exception of Case 1, all cases in the series fell below the minimum normal value of 45 on some occasion. In Case 6, this subnormal value was chiefly due to a chronic nephritis. In Cases 2, 3, and 6 there was a marked tendency of the index to rise as treatment was continued. This rise was coincident with a marked improvement in the clinical condition. Two cases in the series (Cases 7 and 8) showed low indexes during treatment. Case 8 showed a primary rise of the index with a subsequent fall to a still lower level. It will be recalled that this case did not improve with treatment. Case 7, however, showed a marked improvement, and finally the patient went home with compensation reestablished. The two markedly subnormal indexes found in his case are unexplained. There was no evidence of nephritis.

The general tendency of the index to rise during urea feeding does not occur in normal subjects.⁷ It would seem to reflect a general improvement in the clinical condition of the cases under urea treatment. It gives further evidence against the possibility of kidney damage due to urea.

⁷ Austin, J. H., Stillman, E., and Van Slyke, D. D. Factors Governing the Excretion Rate of Urea, *J. Biol. Chem.* 46: 607 (March) 1921.

TABLE 3—Effect of Urea on Urine Volume and Body Weight, the Blood Urea Nitrogen One and a Half Hours After the Second Dose During Time Urea Was Being Given Three Daily, with the Rate of Urea and Urine Excretion at This Time

Case	Disease	Age	Dose of Urea, Gm	Number of Days on Which Urea Was Given	Number of Days Without Urea	Weight at End of Period, Kg	Average Daily Urine Volume, Cc	Chlorid Excretion in 24 Hours as Sodium Chlorid, Gm	Blood Urea Nitrogen per 100 Cc	Rate of Urine Excretion per 24 Hours Calculated from 72 Minute Period, Cc	Urea Nitrogen Excretion Rate per 24 Hours Calculated from 72 Minute Period, Gm	Urea Excretion Index, 10 D	B \sqrt{VW}	Remarks
6	Mitral stenosis, aortic disease (rheumatic), chronic nephritis, ascites, edema of the legs	17				69.8	776	2.20	24.1	1,080	3.14	16.4	16.4	Blood pressure 180 systolic, 90 diastolic
			30	4		67.1	1,129	2.30	43.2	1,700	10.67	24.7	24.7	
			30	4		64.7	1,042	2.39	43.2	1,480				
			45	3		62.4	1,453	2.47	60.9	1,900	10.30	16.0	16.0	
			45	4		61.0	1,416	1.77	86.6	1,420	9.16	13.0	13.0	
			45	3		59.7	1,269		89.0	1,720	14.15	15.8	15.8	
7	Chronic myocarditis, auricular fibrillation, edema of the legs	60				58.9	555		34.3	700	6.09	27.7	27.7	
					4	53.6	226		34.9	560	8.89	44.4	44.4	
						57.6	1,490	12.84	16.6	840	6.57	62.8	62.8	
			30	5		49.6	2,338	9.89	39.8	2,500				
			45	3		48.1	1,569	3.50	53.8	2,100	14.68	37.0	37.0	
			45	4		47.2	1,285	1.00	60.0	1,900	10.05	17.7	17.7	
8	Mitral stenosis, ascites, edema of the legs	17				47.5	726		15.8	960	6.25	58.7	58.7	
					2	48.7	980	1.06	21.0	1,500	3.82	21.6	21.6	
						55.6	344	0.71	21.4	680	6.14	48.6	48.6	Nine days after previous determination
			30	2		53.6	615	1.00	16.9	400	6.69	87.1	87.1	
			30	3		53.5	575	0.33	54.0	880		59.4	59.4	
			45	1		53.1	900	0.46	41.1	1,060	17.99	28.6	28.6	
			45	3		52.9	961	0.52	56.9	1,000	14.41	25.9	25.9	
			60	4		51.9	1,050	0.89	57.4	3,100	18.90	22.6	22.6	
			60	4		51.1	1,115		78.2	2,500	19.99	23.4	23.4	
			60	3					74.9	2,520	19.94			

COMMENT

Many of the most troublesome symptoms of cardiac decompensation are due to the accumulation of fluid in the tissues, in body cavities and in parenchymatous organs. The most important causal factor is certainly the failure of the efficiency of the myocardium, and it is to the amelioration of this that our first efforts must be directed. But frequently in the more advanced cases of heart disease, the maximum efficiency that can be obtained by treatment of the heart *per se* does not suffice to remove edema nor to maintain the patient free from symptoms. In the cases treated with urea a marked improvement in the clinical condition of the patient took place which could be assigned to the action of urea. The treatment succeeded in maintaining an adequate urine output and also in removing edema. As soon as the administration of urea was stopped, the urinary output immediately fell and the clinical condition became worse. When treatment was resumed an improvement again took place. We have studied some of our cases for months and have found that a particular dose will give the same daily urinary output with only slight variations throughout the period investigated. In these cases it has seemed that the maintenance of an adequate water excretion has been instrumental not only in preventing symptoms but also in avoiding a relapse. We have also prescribed its use at home by patients who have been discharged from the hospital after treatment for decompensation. Here again careful treatment of the cardiac condition is of prime importance, but it seems that urea has been of great assistance in keeping these patients comfortable through the maintenance of a normal water balance.

Urea is rapidly absorbed and an increase immediately occurs in the height of the urea in the blood. The mean level of the urea in the blood is dependent on the dosage, and the relationship between them is fairly constant in any particular case. The amount of urea excreted depends on the blood urea, so that with constant urea administration a state of equilibrium is reached between the intake and the output. During urea diuresis the excretion of water runs more or less parallel with that of urea, so that urine volume reflects the concentration of urea in the blood. Undoubtedly the explanation of the diuresis is that the excess of urea circulating in the blood is excreted by the kidney, and during the process carries with it a considerable amount of water.

CONCLUSIONS

- 1 Urea was given in doses of from 30 to 60 gm a day to eight patients with advanced heart failure and was followed by a marked increase in urine volume. The drug was particularly useful in cases in which an adequate water excretion was not maintained after the

edema fluid had been removed by other measures. In some cases it relieved the edema when other remedies had failed.

2 The increase in urine output varied with the dose and followed closely the curve of urea excretion. With continuous administration the daily urine volume was maintained at an almost constant level. The response after administration was rapid, but the effect passed off in a short time unless the dose was repeated.

3 The changes in urine volume and in urea excretion were dependent on the concentration of the urea in the blood.

4 In several of the cases there was a subnormal index of urea excretion which seemed ascribable to advanced heart failure. The index tended to improve with urea administration, along with a general improvement in the clinical condition.

5 Toxic symptoms of any significance did not take place.

6 From these observations we would suggest that urea is a useful diuretic in cases of heart failure with edema in which treatment of the cardiac condition has failed to remove the edema or maintain an adequate water excretion.

DISEASES OF THE LIVER

IV FUNCTIONAL TESTS IN CASES OF CARCINOMA OF THE LIVER AND BILIARY TRACT[†]

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Success in the surgical treatment of carcinoma is attained only when complete removal of the primary malignant lesion is possible. Metastatic dissemination precludes other than palliative measures.

Early diagnosis of intra-abdominal malignant disease, especially of carcinoma involving the hollow viscera, has been greatly facilitated by the roentgen ray. Demonstration of a significant filling defect, especially in the stomach or large bowel, as a rule is possible long before the malignant growth causing such a defect has produced a palpable or visible tumor, and frequently before marked cachexia has developed. The lay conception of cancer as a painful, debilitating, wasting disease is based on clinical pictures of advanced and often incurable malignant disease. The onset in any adult of dyspepsia which resists the usual methods of relief, and remains persistent or progresses, should at least evoke a suspicion of gastric carcinoma until another explanation is found. Similarly, marked or prolonged changes in the activity of the bowels in a person near or past middle life is always suggestive of malignancy of the large bowel. In such a case the probability of carcinoma must be kept in mind until another cause is shown. The suspicion of gastro-intestinal carcinoma must be aroused earlier in the minds of laymen and physicians, and propaganda to correct the pre-roentgen-ray conception of cancer is needed. Assuming early diagnosis and the correspondingly more reasonable chance that the malignant lesion is operable, prognosis will still depend on the presence or absence of metastasis. Palpable implants on the rectal shelf, demonstrable ascites, the rare but deadly button-like infiltration of the umbilicus, roentgenologic evidence of malignant infiltration of the lung, the presence of a "sentinel" gland in the supraclavicular region, or of grossly palpable nodules on the surface of the liver, are all contraindications to radical surgical treatment. Even in the absence of such evidence, laparotomy

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may reveal metastatic nodules in the liver. Any laboratory test, therefore, which may be depended on to disclose the existence of malignant infiltration of the liver, either primary or metastatic, will be of definite clinical value.

Rowntree, Hurwitz and Bloomfield,¹ and more recently Rosenthal,² Ottenberg, Rosenfeld and Goldsmith,³ and Gonzalez and Karr⁴ have reported studies of the phenoltetrachlorophthalein test in cases of carcinoma involving the liver, and have suggested that the test is of particular value in such cases. Their studies, however, were not sufficiently extensive to determine the exact clinical value of the test.

In previous papers,⁵ we have reported a series of tests for hepatic function in patients with obstructive jaundice and in animals with experimentally induced obstruction of the common bile duct. Bilirubinemia and marked retention of phenoltetrachlorophthalein are both characteristically present. Particularly striking was the parallelism between the degree of jaundice and the extent of the dye retention. Included in the latter report was a series of cases of obstruction secondary to tumor of the head of the pancreas, presumably carcinomatous in nature. In these cases, however, the jaundice completely dominated both the clinical picture and the laboratory findings.

We shall now present the results of a study of these tests in three groups of cases. Group 1, fourteen cases of metastatic carcinoma of the liver associated with frank jaundice, Group 2, eighteen cases of metastatic carcinoma of the liver without jaundice, and Group 3, seventeen cases of intra-abdominal carcinoma without demonstrable involvement of the liver.

1 Rowntree, L. G., Hurwitz, S. H., and Bloomfield, A. L. An Experimental and Clinical Study of the Value of Phenoltetrachlorophthalein as a Test for Hepatic Function, *Bull. Johns Hopkins Hosp.* **24**:327-342, 1913.

2 Rosenthal, S. M. A New Method of Testing Liver Function with Phenoltetrachlorophthalein, III, A Clinical Report **79** 2151-2154 (Dec. 23) 1922.

3 Ottenberg, R., Rosenfeld, S., and Goldsmith, L. The Clinical Value of the Serum Tetrachlorophenolphthalein Test for Liver Function, *Arch. Int. Med.* **34** 206-227 (Aug.) 1924.

4 Gonzalez, A., and Karr, W. G. A Comparative Study of the Phenoltetrachlorophthalein and Hemoclastic Tests of Liver Function, *Arch. Int. Med.* **34** 282-291 (Sept.) 1924.

5 Greene, C. H., Snell, A. M., and Walters, Waltman. Diseases of the Liver, I, A Survey of Tests for Hepatic Function, *Arch. Int. Med.* **36** 248-272 (Aug.) 1925. Snell, A. M., Greene, C. H., and Rowntree, L. G. Diseases of the Liver, II, A Comparative Study of Certain Tests for Hepatic Function in Experimental Obstructive Jaundice, *ibid.* **36** 273-291 (Aug.) 1925. Greene, C. H., McVicar, C. S., Rowntree, L. G., and Walters, Waltman. Diseases of the Liver, III, A Comparative Study of Certain Tests for Hepatic Function in Patients with Obstructive Jaundice, *ibid.* **36** 418 (Sept.) 1925.

GROUP 1 METASTATIC CARCINOMA OF THE LIVER ASSOCIATED
WITH FRANK JAUNDICE

In the presence of demonstrated carcinoma elsewhere, jaundice, when present, ordinarily constitutes incontrovertible evidence of involvement of the liver or the biliary tract. Usually it is evidence of obstruction of the extrahepatic biliary passages, although not necessarily so. Table 1 shows the findings with the different tests for hepatic efficiency in a series of fourteen cases of malignant disease of the liver associated with frank jaundice.

Only those positive findings on which conclusions are based are included in the cases reported. The phenoltetrachlorophthalein reading is recorded as for the end of the first hour only. Complete findings are included in the tables. Details of the surgical procedures are not given since the patients were regularly prepared according to Walters' method.

CASE 1 (Table 1) — *Carcinoma of the tail of the pancreas with metastasis to the liver, and jaundice without obstruction to the biliary passages*

A man, aged 58, came to the Mayo Clinic, Nov 17, 1924, because of marked loss of weight and extreme jaundice of four weeks' duration. In August, 1924, he had first noticed a dull pain localized in the right epigastrium, which was accompanied by nausea and relieved by vomiting. He had had two or three attacks of pain, which was never severe or colicky. Jaundice had developed four weeks before admission, it fluctuated in intensity at the onset. The stools were light and the urine dark. Moderate pruritus was present. Jaundice, constipation, weakness, malaise, dyspnea, anorexia and nausea were increasingly evident. During this period he lost 52 pounds (23.6 kg) although he still weighed 254 pounds (115.2 kg).

The liver was enlarged and tender, extending 6 cm below the costal margin. The surface was smooth and the edge sharp. Fluoroscopic examination of the stomach showed an extragastric mass. No glandular enlargement was found. The urine contained much bile and a trace of albumin. The serum bilirubin was 71.3 mg. Retention of phenoltetrachlorophthalein was extreme, the one hour reading being 51 per cent. The patient failed rapidly and progressively. Hematemesis appeared November 25, and he became comatose. The blood urea became elevated to 68 mg two days before death, November 27.

At necropsy, carcinoma of the tail of the pancreas with extensive metastatic involvement of the liver, kidneys and lungs was found, but curiously enough there was no mechanical obstruction to the extrahepatic biliary passages. This case is of unusual interest in showing the possibility of the development of an extreme degree of jaundice in the absence of demonstrable obstruction to the extrahepatic biliary passages.

CASE 3 (Table 1) — *Primary carcinoma of the bile ducts with involvement of the liver and obstructive jaundice*

A man, aged 60, came to the Mayo Clinic, May 6, 1924, because of attacks of abdominal pain and of jaundice of one month's duration. For the preceding year he had suffered from epigastric fulness and distress after meals. One month before admission and following a rough automobile ride, he had an attack of severe, disabling pain in the upper part of the abdomen, accompanied by nausea and followed by soreness along the right costal margin. He subsequently had four or five similar attacks, each of approximately five minutes' duration. Jaundice appeared after the second of these attacks.

He had lost 15 pounds (6.8 kg) during the three weeks preceding his admission. Marked jaundice was present. The liver edge was palpable 4 cm below the costal

margin and a rounded mass, approximately 10 cm in diameter, was felt below the liver edge. There was a suggestion of free fluid in the abdomen. Urinalysis revealed bile 3, but no albumin or casts. The serum bilirubin was 43.2 mg. There was marked retention of phenoltetrachlorophthalein, with a reading of 33 per cent. During the twelve days the patient was in the hospital, the jaundice decreased from 4 to 2. Bile appeared in the stools in small amounts at this time. June 11 the serum bilirubin was 26.5 mg. The phenoltetrachlorophthalein reading was 26 per cent.

At operation, June 18, an inoperable carcinoma, evidently primary in the bile ducts, was encountered. There was marked involvement of the liver and of the hepatic, common and cystic ducts. The gallbladder was large and distended, but was not opened.

The similarity between the history and the symptoms in this case to those in cases of stone is marked, and a clinical diagnosis of obstructive jaundice due to stone in the common duct was made. While the patient was under observation, the jaundice decreased and a small amount of bile was present in the stool. A similar decrease in the degree of jaundice while the patient was under observation was seen in Case 5. It is evident that such changes cannot be interpreted as pathognomonic of obstruction due to stone. This case is also of interest because of the similarity between the fall in the serum bilirubin and the degree of dye retention which accompanied the temporary appearance of bile in the stool on the one hand, and the improvement seen in cases of obstructive jaundice following the operative relief of the obstruction on the other. This illustrates the preponderant effect of the jaundice and bile retention on the functional findings in the cases of carcinoma with obstruction to the biliary passages.

CASE 6 (Table 1)—Carcinoma of the head of the pancreas with involvement of the liver and obstructive jaundice

A man, aged 34, was admitted to the Mayo Clinic, August 6, 1924, because of painless jaundice of ten weeks' duration. There was a history of a questionable chancre fifteen years before, but the Wassermann reaction was negative. For three months previous to the onset of the jaundice, the patient had been drinking excessively. Two days before, he noticed that the urine was dark and the stools light. The stools later became clay colored. He consulted a physician who gave him three injections of neoarsphenamin without the jaundice being affected. Bile was obtained by duodenal drainage at that time.

The patient's weight on admission was 116 pounds (52.6 kg), representing a loss of 30 pounds (13.6 kg) in three months, and extreme jaundice was present. There were no palpable lymph nodes. The liver was enlarged, the edge being palpable at the level of the umbilicus, the edge was sharp and the surface smooth. No nodules were felt. The gallbladder and spleen were not palpable. The urine contained albumin, casts and bile. The stools were clay colored, and tests for bile were negative. The guaiac test for blood in the stools was occasionally positive. The serum bilirubin was 36.4 and there was marked retention of phenoltetrachlorophthalein, the reading being 24 per cent. Duodenal drainage was attempted on three occasions, there was no return of bile though traces of blood were obtained.

The patient was kept under observation for ten days, during which time the pulse and temperature were normal. Thereafter the course was strikingly febrile, with repeated chills and a temperature varying between 102 and 104 degrees. The anemia increased rapidly, the hemoglobin falling to 35 per cent. A transfusion was given without benefit. The stool remained acholic, but the serum bilirubin

TABLE 1—Carcinoma of

Case	Date	Age, Years	Sex	Edge of Liver Palpable, Cm	Jaundice	Duration of Jaun- dice, Months	Urine			Blood Count			Phenolsulphone- phthalein, per Cent	Blood Nitrogen Partition, Mg per Cent							
							Specific Gravity	Albumin	Casts	Bile Pigments	Hemoglobin, per Cent	Erythrocytes, Millions		Leukocytes	Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino-Acid Nitrogen		
							Diagnosis	Confirmed	Pathologically												
1	11/17/24	53	M	6	4	1	1 012	1	0	4	65	4 03	16,200		34	2 6	2 0				
2	12/ 6/24	61	M	*	4	1	1 023	3	0		70	4 23	15,000		22	1 7					
3	5/30/24	60	M	4	4	1	1 012	0	0	3	71	4 41	6,500		32	23	2 2	1 6	6 0		
4	6/11/24 6/22/23	49	F	0	2	1	1 017	3	1	2	75	4 50	10,700	20	33	54 16	4 1	1 1 2 7	5 1 6 7		
5	11/26/23 12/ 5/23	59	F	4 4	4 2	2 wk	1 010 1 006	2 1	0 0	+	73	4 80	26,200 14,800	45 60	28 14	3 1	1 4				
6	8/ 9/24	36	M	15	4	3	1 016	2	1	3	60	3 70	8,250	27	26	20	1 6	1 5	4 8		
7	6/23/23	67	M	*	2	13	1 021	1	0		85		10,900		32	3 5	2 2	6 5			
							No	Pathologic			Diagnosis		Made								
8	9/29/24	70	F	*	4	1	1 007	1	1	3	73	4 87	14,700		27	33	2 1	1 8	6 1		
9	10/30/24	61	M	6	3	2 wk	1 025	1	0	3	73	4 59	7,000		29	13	1 7	1 7	5 7		
10	8/15/24	63	M	†	2	1	1 025	1	0	1	83	4 14	8,600	80	33	33	2 6	1 8	5 2		
11	3/ 5/25	46	M	5*	4	6	1 016	1	0		80			80	20	15					
12	3/31/24	74	M	5*	4	1	1 040	3	3	3	62		3,800		29	34		1 4	5 4		
13	3/ 5/24	60	F	8*	4	4	1 016				58	3 24	9,800	80	24	11					
14	5/26/24	45	M	2*	3	2	1 025	2		4	90	4 20	5,800	20	26			2 5	5 1		

* Large and nodular

† Ascites prevented palpation

TABLE 2—Carcinoma of

Case	Date	Age, Years	Sex	Edge of Liver Palpable, Cm	Jaundice	Urine			Blood Count			Phenolsulphone- phthalein, per Cent	Blood Nitrogen Partition, Mg per Cent				
						Specific Gravity	Albumin	Bile Pigments	Hemoglobin, per Cent	Erythrocytes, Millions	Leukocytes		Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen
Diagnosis Confirmed Pathologically																	
15	6/11/24	73	M	0	0	1 028	1		72	4 03	6,000		33	46	2 4	1 7	5 1
16	7/ 2/24	46	F	8	0	1 022	0	+	63	3 80	19,200		27	35	3 0	2 0	5 4
17	12/ 6/24	42	F	*	0	1 015	4	0	73	4 52	7,100		28	22	3 3	1 3	4 9
18	8/ 9/24	72	M	*	†	1 019	1		80	4 08	22,300	15	276	466	6 5	6 3	8 7
19	5/ 3/24	56	M	0	0	1 010	1	0	67		12,700	45	29	28	2 8	1 6	6 8
20	3/ 5/25	55	M	*	0	1 018	0	0	19	1 61	6,900			62		1 4	
21	12/ 8/24	48	M	0	0	1 027	0	0	43	2 91	8,900		35	27		1 2	5 7
22	12/ 8/24	60	M	0	0	1 027	0	0	73	4 44	8,400		33	12	3 4	1 2	6 8
23	1/12/25	30	M	0	0	1 028	2	0	68	4 31	10,900			25	2 5	1 1	
24	10/24/24	36	M	0	0	1 027	0	0	64	4 56	21,000		29	27	3 0	1 6	6 3
25	2/ 9/25	70	M	0	0	1 025	0	0	25	2 67	5,700			42	3 3	1 2	
No Pathologic Diagnosis Made																	
26	11/31/23	70	M	*	0	1 026	1		80		9,800						
27	2/28/24	66	M	†	†	1 022	1	0	80	4 15	7,500	55	34	36	3 0	1 6	
	3/13/24			†	†	1 015	0						33	44			
28	3/ 4/24	58	F	15	†	1 017	0		77	4 27	6,500		33	31			
29	3/ 9/25	73	M	†	0	1 027	0	0	70	4 32	7,800			28	2 9	2 1	
30	11/14/24	45	F	*	0	1 010	1	0	43	3 54	15,800	45		16	2 7	1 6	
31	11/28/24	39	M	*	0	1 027	1		60	3 62	17,400	65	31	32	2 4	1 4	5 5
32	3/ 9/25	64	M	*	0	1 024	1		51	4 14	6,800		52	52	3 0	2 0	

* Liver enlarged and nodular

† Liver palpable

Bile Index	Bile Pigments (Serum)		Coagulation Time		Fructose Tolerance Blood Sugar, Mg per Cent				Phenol-tetrachlorophthalein Dye in Serum, per Cent			Dye in Urine, Mg	Remarks
	Serum Bilirubin, Mg per Cent	Van den Bergh Direct Reaction	Minutes	Seconds	Normal	1 Hour	2 Hours	Change	15 Minutes	1 Hour	2 Hours		
	71.3	+	14	30						51	45	15	Died, carcinoma of tail of pancreas, metastasis to liver
	22.3	+							34	36	30	7.0	Carcinoma of hepatic duct, extension to liver
330	43.2	+	12		95	137	130	42	38	33	22		Carcinoma of bile ducts, involvement of liver
142	26.5	+	10	15	86	105	99	19	28	26	26	11.9	After spontaneous decrease in jaundice
									28	30	28	1.5	Adenocarcinoma of gallbladder, involvement of liver
25			11	50	117	140	145	28		30	25	5.0	Carcinoma of liver, marked jaundice
18	1.9		9							12	11	0.3	After spontaneous decrease in jaundice
210	35.4	+	13		90	121	129	34	24	24	20	10.8	Died, carcinoma of head of pancreas, metastasis to liver
			6	11					15	20	13	1.5	Carcinoma of liver, biliary fistula with partial obstruction
													No Pathologic Diagnosis Made
	25.2	+			86	140		54	44	34			Carcinoma of pancreas, diabetes, metastasis to liver
	20.2	+	7	30					40	36	8.5		Malignant rectal polyp, metastasis to rectal shelf and liver
84	10.0	+							27	34	34	1.8	Inoperable carcinoma with ascites, metastasis to rectal shelf
100	15.6	+	16	20	103	117	115	14	30	32	22	0.9	Carcinoma of pancreas, metastasis to liver
120	36.2	+	12		93	192	200	107	30	28	24	8.0	Carcinoma of pancreas, diabetes, metastasis to liver
100	21.5	+			176	184	180	8		25	20	2.4	Carcinoma of pancreas, diabetes, metastasis to liver
			7						23	20	15	6.0	Carcinoma of liver

Bile Pigments (Serum)			Fructose Tolerance Blood Sugar, Mg per Cent				Phenol- tetrachlor- phthalein Dye in Serum, per Cent			Dye in Urine, Mg	Remarks
Bile Index	Serum Bilirubin, Mg per Cent	Van den Bergh Direct Reaction	Normal	1 Hour	2 Hours	Change	15 Minutes	1 Hour	2 Hours		
12	2.8	0					18	17	14	0.0	Carcinoma of rectum, metastasis to liver
12	1.6	++	100	127	110	27	18	17	11	0.0	Carcinoma of gallbladder, metastasis to liver
	0.9	0					12	8	3	0.0	Multiple tumors of liver, possibly sarcoma
	5.2	+					12				Carcinoma of stomach, peritoneal carcinomatosis, metastasis to liver
4	1.1	0						7	2	0.0	Recurring carcinoma of rectum, metastasis to liver
	0.2	0					8	6	2	0.2	Carcinoma of stomach, metastasis to liver
	0.2	0					6	4	2	0.0	Carcinoma of stomach, invasion of liver
	0.5	0					6	3	—	0.0	Carcinoma of colon, metastasis to liver
	0.1	0					6	2	—	0.0	Carcinoma of rectum, metastasis to liver
6	1.0	0					5	2	—	0.0	Carcinoma of colon, metastasis to liver
	0.1	0					5	2	—	0.0	Carcinoma of stomach, metastasis to liver
							No	Pathologic	Diagnosis	Made	
7	1.2	0						28	28	0.3	Carcinoma of liver
13	2.4	0	99	87	99	12	19	22	18	0.7	Carcinoma of stomach, probable metastasis to liver
16	2.6	0					12	22	22	1.3	Carcinoma of liver
	1.4	0	90	137	125	38	12	12	8	0.3	Carcinoma of colon, probable metastasis to liver
	0.1	0						11	8		Adenocarcinoma of ovary, abdominal carcinomatosis, probable metastasis to liver
	1.1	0						7	3		Carcinoma of liver
	1.1	0					7	5	3	0.2	Carcinoma of hepatic flexure, probable involvement of liver
	0.2	0					6	3	1	0.0	

decreased to 162 mg and the jaundice decreased to a corresponding degree. The toxemia, however, increased and during the last week the blood urea became elevated, reaching a level of over 200 mg the day before death, September 9. During the last three days of life, a mass became palpable below the liver edge and was believed to be the gallbladder, but operation was not considered. At necropsy carcinoma of the head of the pancreas with obstruction of the common bile duct, metastasis to the liver and biliary cirrhosis were found.

This case presented many angles from the standpoint of diagnosis which was definitely made only at necropsy. The history is given in detail to emphasize the extreme difficulty of differential diagnosis in many of these cases. Catarrhal jaundice was first considered but did not account for the duration and intensity of the jaundice. The appearance of chills and fever with a moderate leukocytosis suggested the presence of cholangitis. The possibility of cirrhosis was emphasized by the history of alcoholism. There was a questionable history of syphilis, but the negative Wassermann reaction and the lack of response to antisyphilitic treatment justified its exclusion. The extreme prostration and toxemia in the later stages of the disease resembled acute yellow atrophy. Carcinoma was considered, but in view of the patient's age and the absence of a demonstrable primary growth or of metastatic lesions it was thought to be unlikely. The final clinical diagnosis was obstructive jaundice with probable hepatitis, cirrhosis and cholangitis. In this instance the laboratory findings were of no aid in differential diagnosis, for the high serum bilirubin and marked dye retention were not to be differentiated from those seen in obstructive jaundice due to stone or stricture of the common duct.

GROUP 2 METASTATIC CARCINOMA OF THE LIVER WITHOUT JAUNDICE

Rolleston⁶ reports that in a series of cases of secondary malignant disease of the liver, the primary carcinoma in over 75 per cent was situated in the stomach, colon, esophagus, pancreas or gallbladder. In the absence of obstruction to the bile duct, jaundice is uncommon and the clinical diagnosis of hepatic involvement by the malignant process is dependent largely on rapid and progressive enlargement, and particularly nodular enlargement, of the liver. Table 2 shows the changes in a series of eighteen cases of this type. In this group the laboratory tests are of the greatest clinical value.

CASE 15 (Table 2) — *Carcinoma of the rectum with metastatic nodules in the liver*

A man, aged 73, was admitted to the Mayo Clinic, April 28, 1924, because of diarrhea and rectal pain of one year's duration. Slight bleeding had been observed for three or four months.

⁶ Rolleston, H. D. *Diseases of the Liver, Gallbladder and Bile Ducts*, Ed. 2, London, Macmillan Company, 1924.

His weight on admission was 153 pounds (69.4 kg), representing a loss of 17 pounds (7.7 kg) in about four months. There was no jaundice, and no abdominal masses or tenderness were present. The liver was not palpable. A mass could be felt in the rectum, and on proctoscopic examination a large, fixed, partially obstructive lesion of the upper rectum and lower sigmoid was found. The serum bilirubin was 2.8. There was moderate retention of phenoltetrachlorophthalein with a reading of 17 per cent.

At operation a large carcinoma of the rectum was found. No definite local glandular involvement could be demonstrated, but there was extensive involvement of the liver with a large metastatic nodule on the superior anterior surface of the right lobe. On account of the obstruction a permanent colostomy was made.

In this case the constitutional symptoms were those of carcinoma, but there were no local signs to suggest invasion of the liver. The laboratory findings of a moderate dye retention and slight bilirubinemia suggested the extensive metastatic involvement of the liver revealed by operation.

CASE 16 (Table 2) —*Carcinoma of the gallbladder with invasion of the liver*

A woman, aged 46, came to the Mayo Clinic, June 4, 1924, because of recurring attacks of pain under the right costal margin, accompanied by fever of eight months' duration. She had had typhoid fever in 1915. In November, 1923, she had an attack of severe pain in the right upper quadrant radiating to the lower abdomen. She was in bed for two or three days, during which time an egg-shaped mass was observed below the costal margin. The mass was continuously present after January, 1924, and the liver edge likewise became palpable. In May the liver was definitely enlarged, daily fever up to 102 degrees was observed, and the patient became much weaker and complained of a feeling of epigastric weight and discomfort after eating.

On admission the patient was very weak and emaciated. She weighed 106 pounds (48.1 kg), having lost 20 pounds (9 kg) during the last two months. There was no jaundice. The liver edge was felt 8 cm below the costal margin, while a rounded mass extended 6 cm below the liver margin. No glandular enlargement or palpable evidence of metastasis was found. The urine was normal except for the presence of a trace of bile. A test of the stool for bile was negative. The serum bilirubin was 1.6 mg. There was moderate retention of phenoltetrachlorophthalein, as the reading was 17 per cent. The patient was under observation ten days, during which time the temperature was irregularly elevated to a maximum of 102 degrees.

At operation, July 2, gallstones, carcinoma of the gallbladder and multiple metastatic nodules in the liver were found. It seemed likely that the fever was produced by infection in the gallbladder, but the extensive invasion of the liver and the weakened condition of the patient precluded further operative intervention. The pathologic diagnosis of a specimen removed from the liver was adenocarcinoma.

In this case the symptoms were preponderantly those of cholelithiasis with added infection. The laboratory tests, however, showed the presence of definite dye retention.

CASE 17 (Table 2) —*Multiple tumors of the liver*

A woman, aged 42, was admitted to the Mayo Clinic, Dec. 6, 1924, because of a progressively increasing epigastric mass which she had noticed two months before. It was not painful although she had a certain sense of epigastric weight and discomfort. She had lost 15 pounds (6.8 kg) because of voluntary dietary restriction although there was no definite food intolerance.

She was sallow but not icteric in appearance. The liver was greatly enlarged. There was a firm projecting mass in the right epigastrium, which appeared to be from 8 to 10 cm in diameter. Smaller nodules were felt over the remainder of the surface of the liver, and there was a tumor in the pelvis 8 cm in diameter. There was no enlargement of the superficial lymph nodes and a roentgenogram of the chest did not show metastatic invasion. The urine

contained albumin 3, but no bile. The serum bilirubin was 0.9 mg. There was moderate retention of phenoltetrachlorophthalein with a reading of 8 per cent.

An exploratory laparotomy was performed, December 17. There was one large, rounded solid tumor in the right lobe of the liver and several smaller tumors. It did not seem wise to remove a portion of the tumor for histologic examination, but the gross appearance was that of a sarcoma rather than that of a carcinoma. No primary source of the growth could be found in the abdomen. The pelvic mass was a dermoid of the left ovary.

The short history and the large nodular liver led to the clinical diagnosis of probable tumor of the liver. The moderate degree of dye retention furnished additional evidence in support of this diagnosis, which was confirmed by the findings at operation.

CASE 21 (Table 2) — *Carcinoma of the stomach with direct extension to the liver*

A man, aged 48, was admitted, Dec 8, 1924, because of stomach trouble of eighteen months' duration. Obstructive symptoms associated with progressive loss of 40 pounds (18.1 kg) in weight, weakness and anemia developed.

The patient was anemic and cachectic in appearance. There was no jaundice, and the superficial lymph nodes were not enlarged. The liver was not palpable but there was a firm, fixed, nodular mass, apparently confluent with the stomach, in the left epigastrium. Gastric analysis revealed marked retention with a fasting content of 1,020 cc. The total acidity of this fluid was 60 and of the free acids 0. Roentgenograms showed an obstructing lesion in the stomach. The serum bilirubin was 0.2. There was no retention of phenoltetrachlorophthalein, the reading was 4 per cent.

At operation a carcinoma of the stomach was found with extensive involvement of the adjoining lymph nodes. The carcinoma had perforated against the liver, extending locally into its substance. Resection of the entire growth was not possible.

There were no direct clinical evidences of hepatic involvement in this case, and the laboratory tests were negative. The carcinoma had invaded the liver by direct extension rather than by the blood or lymph stream. It is probable that the invasion was strictly localized and much less extensive than usual, this might explain the negative laboratory findings.

CASE 24 (Table 2) — *Carcinoma of the descending colon with metastatic nodules in the liver*

A man, aged 36, was admitted to the Mayo Clinic, Oct 20, 1924, because of constipation and left sided abdominal pain of ten months' duration. Blood clots had been passed by bowel on two or three occasions.

The patient was cachectic in appearance. His weight was 117 pounds (53.1 kg), representing a loss of 43 pounds (19.5 kg) in ten months. There was an enlarged gland in the right supraclavicular fossa. The liver edge was just palpable at the costal margin. Roentgenograms of the colon revealed a filling defect in the upper portion of the descending colon near the splenic flexure. The serum bilirubin was 1 mg. There was no retention of phenoltetrachlorophthalein, the reading was 2 per cent.

The supraclavicular gland was removed for examination and found to be inflammatory. At exploratory laparotomy a carcinoma of the descending colon was found, the glands along the spine were involved, and large and small carcinomatous nodules were present in the liver. Because of the extensive metastasis removal of the primary growth was not possible.

The patient's constitutional symptoms were those of neoplastic disease, but there were no direct clinical signs to indicate metastasis, and the laboratory findings were negative. There was neither bilirubinemia nor dye retention.

CASE 26 (Table 2)—*Carcinoma of the liver of unknown origin*

A man, aged 70, was admitted to the Mayo Clinic, Nov 1, 1924, because of dull epigastric pain of three months' duration. The pain usually came on one hour after eating, was confined to the epigastric and umbilical regions, and was not severe. Food or a bowel movement gave temporary relief. He had lost about 25 pounds (11.3 kg) during the preceding three months. There was no jaundice.

There was no enlargement of the superficial lymph nodes. The liver was not palpable. The urine contained a trace of albumin. Roentgenograms of the stomach, colon and intestines failed to reveal any source of malignant disease. While the patient was under observation in the hospital, a mass became palpable in the epigastrium. It rapidly increased in size until it could readily be identified as the liver. November 21, there was marked retention of phenol-tetrachlorophthalein with a reading of 28 per cent. The liver now extended to the umbilicus, and appeared to be nodular. Surgical intervention was not indicated.

The clinical diagnosis was carcinoma of the liver, although the primary focus was not identified. The laboratory studies demonstrated marked dye retention, a finding in complete accord with the clinical diagnosis.

GROUP 3 INTRA ABDOMINAL CARCINOMA WITHOUT DEMONSTRABLE INVOLVEMENT OF THE LIVER

As a control of the cases reported in Tables 1 and 2, a series of seventeen cases of carcinoma of the stomach or colon without demonstrable involvement of the liver is presented (Table 3). In these cases the surgeon found the liver normal at the time of operation, which does not entirely exclude the presence of carcinomatous nodules since they may be either completely buried within the substance of the liver or, if external, so situated as not to be apparent at the time of operation. In either case, the degree of hepatic involvement would presumably be small. Three of the cases show a departure from the normal by the laboratory tests.

CASE 33 (Table 3)—*Carcinoma of the ascending colon*

A man, aged 41, was admitted to the Mayo Clinic, June 6, 1924, because of attacks of colicky epigastric pain of nine months' duration. A month before admission he began to suffer from diarrhea, and clotted blood was observed in the stools. He had lost 30 pounds (13.6 kg), and anemia became progressively more marked during the course of his illness.

He was weak and anemic in appearance. There was no jaundice and the liver was not palpable, but there was a poorly defined tender mass in the cecal area extending upward toward the costal margin. The superficial glands were not enlarged. Roentgenograms of the colon showed a filling defect in the ascending colon. The serum bilirubin was 1.9 mg. There was moderate retention of phenoltetrachlorophthalein with a reading of 10 per cent.

At operation a carcinoma of the posterior wall of the ascending colon which involved the cecal edge of the ileocecal valve was removed. There was local glandular involvement, but there were no visible evidences of invasion of the liver. The growth was removed, the patient recovered without complication, and his present condition is satisfactory.

CASE 34 (Table 3)—*Malignant rectal polyp*

A physician, aged 47, was admitted to the Mayo Clinic, Nov 15, 1924, because of blood in the stools. In 1909 varicose veins had been removed under chloroform anesthesia, following the operation he was jaundiced for several days but recovered completely. Blood had been noted in the stool six months before admission.

TABLE 3—*Carcinoma*

Case	Date	Age, Years	Sex	Urine		Blood Count			Phenolsulphone- phthalein, per Cent	Blood Nitrogen Partition, Mg, per Cent				
				Specific Gravity	Albumin	Hemoglobin, per Cent	Erythrocytes, Millions	Leukocytes		Nonprotein Nitrogen	Urea	Uric Acid	Creatinin	Amino Acid Nitrogen
33	6/16/24	41	M	1 023	0	40	2 72	7,100	50	55	76	2 9	1 4	61
34	11/19/24	47	M	1 027	0	75	4 47	8,100		37	32	2 8	1 7	62
35	4/11/24	67	F	1 027	1	70	4 03	5,200		25	21	2 4	1 4	63
36	9/11/24	56	M	1 019	1	34	3 01	5,600		28	24	3 3	1 8	58
37	10/17/24	59	M	1 016	0	40	3 22	7,400	30	28	29	3 5	1 8	82
38	4/ 3/24	58	M	1 022	1	50	3 16	6,500	60	35	45	3 3	1 6	57
39	11/ 7/23	59	M	1 020	1	46	3 40	9,000			36	2 8	2 9	
40	4/25/24	48	M	1 011	0	71	4 09	5,800		37	50		1 7	69
41	24	43	M	1 030	1	67	3 97	5,200		38	23	4 4	1 7	64
42	11/ 2/23	47	M	1 019	0	45	3 33	10,500			38	3 1	2 4	
43	5/22/24	64	M	1 023	2	62	3 96	11,200	55	33	29	1 9	1 8	54
44	11/ 8/23	67	M	1 022	0	50	3 50	5,600	55		21			
45	6/ 2/24	60	M	1 027	1	72	4 28	5,100		29	39	2 1	1 8	62
46	8/ 2/24	76	M	1 027	0	75	4 32	8,000		38	31	2 2	1 8	
47	9/ 3/24	55	M	1 035	0	73	4 25	8,000		32	38	2 0	1 6	58
48	10/24/23	59	M	1 022	0						65			
49	10/15/23	48	F	1 022	1	75	4 51	10 300			23	4 8	2 2	

The patient was obese, weighing 243 pounds (110.2 kg). There was no jaundice. The edge of the liver was palpable at the costal margin and was sharp. There were no superficial glandular enlargements. Proctoscopic examination revealed a large rectal polyp. The serum bilirubin was 2.2 mg. The dye reading of 7 per cent indicated a slight retention of phenoltetrachlorophthalein.

At operation a carcinomatous polyp of the rectum was removed through a Kraske incision. There was no local glandular involvement. Direct examination of the liver was not possible.

In this case there was slight dye retention and bilirubinemia suggestive of hepatic disturbance. The rectal polyp was malignant, but there was no local evidence of extension of the growth. It is likely that the patient's jaundice in 1909, following chloroform anesthesia, had initiated changes which were responsible for the functional disturbances, although the patient had been symptomless since then. On the other hand, invasion of the liver cannot be ruled out.

CASE 42 (Table 3)—*Carcinoma of the stomach, secondary anemia*

A man aged 47, was admitted to the Mayo Clinic, Nov. 2, 1923. In February he had begun to have sharp abdominal pains shortly after eating. The liver and spleen were not palpable. The gastric acidity was normal, but old blood was present in the specimen. Roentgenograms disclosed carcinoma of the stomach high on the posterior wall. The phenoltetrachlorophthalein reading was 4 per cent at the end of an hour. At exploration extensive local glandular involvement was found and the carcinoma was not removed. There was no evidence of involvement of the liver.

CASE 48 (Table 3)—*Carcinoma of the stomach*

A man, aged 59, was admitted to the Mayo Clinic, Oct. 24, 1923. Six months before he had begun to have dull abdominal pain from one to two hours after eating, and he had lost weight and strength progressively. Retention vomiting had begun about a week before admission.

Bile Pigments (Serum)			Fructose Tolerance Blood Sugar, Mg per Cent				Phenol tetrachlor- phthalein Dye in Serum, per Cent			Dye in Urine, Mg	Remarks
Bile Index	Serum Bilirubin, Mg per Cent	Van den Bergh Direct Reaction	Normal	1 Hour	2 Hours	Change	15 Minutes	1 Hour	2 Hours		
6	14	—	101	125	124	24	11	10	5	11	Carcinoma of ascending colon, local glandular involvement
12	19	—	90	122	93	32	11	7	5	08	Carcinomatous polyp of rectum
7	06	—					11	6	3	03	Carcinoma of stomach, inoperable, local glandular involvement
8	05	—					10	5	2	05	Carcinoma of cecum
9	01	—	87	122	135	48	10	4	1	06	Colloid carcinoma of stomach, peritoneal metastasis
16			105	124	92	19	8	4	1	06	Carcinoma of stomach, operable, no glandular involvement
5	03	—	80	81	92	12	7	4	2	04	Carcinomatous tumor of epigastrium
6	10	—	87	106	94	19	7	4	1		Carcinomatous ulcer of stomach, no glandular involvement
11	20	—	87	108	107	21	7	3	1	04	Carcinoma of stomach, marked glandular involvement
6	06	—					7	3	—	04	Carcinoma of stomach, inoperable
13	06	—	107	87		20	7	2	—	02	Carcinoma of stomach, slight local glandular involvement
							6	2	—		Tumor of stomach
							5	3	—	05	Carcinomatous ulcer of stomach, no glandular involvement
								3	—		Carcinoma of pylorus, no glandular involvement
							5	2	—	00	Carcinoma of rectosigmoid
							4	—	—	00	Carcinomatous gastric ulcer
							4	—	—	00	Carcinoma of stomach

On examination the patient was emaciated, he had lost 40 pounds (181 kg) and was weak. The liver could not be palpated but there was a movable mass in the right upper abdominal quadrant. A test meal revealed a twelve-hour gastric retention of 1,150 cc, and carcinoma at the pylorus was evident in the roentgenograms. The phenoltetrachlorphthalein reading was 2 per cent.

At operation a freely movable carcinomatous gastric ulcer involving the pyloric end of the stomach was found. There was no evidence of metastasis. A partial gastrectomy of the posterior Polya type was performed. The post-operative recovery was excellent.

INDIVIDUAL TESTS

Fructose Tolerance—It has been reported that a lowered tolerance for carbohydrates accompanies many cases of carcinoma. Friedenwald and Grove⁷ have recently reviewed the literature on this subject and found an apparently constant lowering of the glucose tolerance in a small series of cases of gastro-intestinal carcinoma. Kelly,⁸ on the other hand, did not find significant changes in the glucose tolerance in his series of cases. The blood sugar in normal persons is less elevated following the ingestion of fructose than it is after the ingestion of an equal amount of glucose. A reduction in the fructose tolerance has

7 Friedenwald, J, and Grove, G. H. The Blood Sugar Tolerance Test as an Aid in the Diagnosis of Gastro-Intestinal Cancer, *Tr. A. Am. Phys.* **35** 97, 1920.

8 Kelly, T. C. Blood Sugar Retention in Carcinoma, *Am. J. M. Sc.* **169** 216 (Feb.) 1925.

been assumed, but not proved, to be a specific index to disturbances in the glycolytic activity of the liver

In our series the fructose tolerance was definitely diminished in three cases and suggestively lowered in three, in a total of eight tests on patients with carcinoma involving the liver and associated with jaundice. Clinical diabetes also was present in two of the cases, showing a reduced carbohydrate tolerance. Tests were made on three patients with carcinoma involving the liver without jaundice, the fructose tolerance was reduced in one. In those patients with carcinoma without proved metastasis to the liver there were two with a lowered fructose tolerance in a total of eight. Because of the irregularity with which positive tests were secured and the frequent association of pancreatic disease and clinical diabetes with the disturbance of the liver in this series of cases, we question the clinical value of the fructose tolerance test as a specific clinical index to the functional efficiency of the liver.

Blood Sugar Level—Friedenwald and Grove report an increase in the fasting blood sugar level in the cases of carcinoma studied by them. They also quote previous workers who made similar observations. In our series of cases, there was no significant elevation of the fasting blood sugar above the normal. A high reading, 176 mg per cent, was obtained in only one instance, and in that case there were clinical evidences of diabetes secondary to a carcinoma of the head of the pancreas.

Nitrogen Partition of the Blood—There were no changes in the nonprotein nitrogen of the blood that could be attributed directly to the carcinomatous process. In five cases the blood urea was greater than 50 mg. In one case (Case 18, Table 2), there was an associated pyelonephritis and a terminal increase in the blood urea from 70 to 466 mg. In another (Case 6, Table 1), an increase in the blood urea from 20 to 200 mg was associated with the development of a fatal toxemia, and was not affected by the intravenous administration of glucose. Three of the patients with carcinoma had a blood urea level of 50 mg or more on admission. In all, however, this was associated with partial intestinal or pyloric obstruction with consequent dehydration, and the blood urea promptly returned to normal following the administration of an adequate amount of sodium chlorid solution. The general level of the blood urea was perhaps slightly lower in those cases with jaundice, 25.5 mg, than in Groups 2 and 3, from 29.3 to 36.4 mg, although all were within the lower limits of normal. The changes, however, were not sufficient to be of clinical significance in any individual case.

Noteworthy changes were not observed in the other nonprotein nitrogenous constituents of the blood studied. The total nonprotein nitrogen, uric acid, creatinin ammonitrogen all came within the limits of normal.

Bile Pigments—The cases were arbitrarily grouped according to the presence or absence of clinical jaundice. When icterus was manifest the serum bilirubin was uniformly increased, the highest reading being 71.6 mg (Table 1). In all these cases the icterus index was increased and the direct van den Bergh reaction was obtained, the findings being entirely comparable to those in the series of cases of obstructive jaundice previously reported.

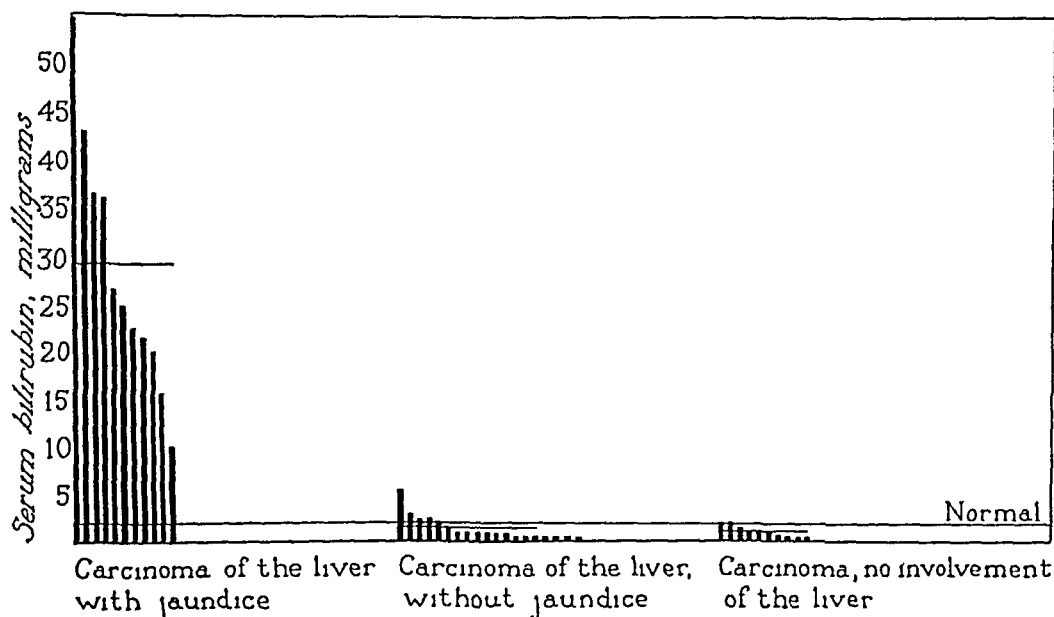


Fig 1—Variation in the serum bilirubin in the different types of carcinoma studied. The maximal normal level is indicated, and in each group the average value is shown by the horizontal line.

Normal values for the serum bilirubin were obtained in the third group of carcinoma cases, those without evident metastatic involvement of the liver. Latent icterus was demonstrable in three of the eighteen cases of carcinoma with metastatic involvement of the liver reported in Table 2. In one the serum bilirubin was 5.6 mg and the direct van den Bergh reaction was obtained. The skin and sclerotics of this patient had a subicteric tint. In the other two patients, clinical evidences of jaundice were wanting. The serum bilirubin readings were normal in the other cases of this group.

Phenoltetrachlorophthalein—We have previously shown the consistent occurrence of a marked retention of phenoltetrachlorophthalein both in patients with obstructive jaundice and in animals following ligation of the common bile duct. In such cases the bile retention

apparently conditions the dye retention. The results of the test in patients with carcinomatous involvement of the liver and jaundice were similar to those in obstructive jaundice of any origin. Retention of dye in the blood stream was marked. In this series of cases, the maximal reading in the one-hour sample was 51 per cent and all the cases showed a reading of 20 per cent or over. This is in marked contrast to the maximal normal reading of 3 per cent.

The second group of cases is more striking even than the first. In a large proportion of these cases (eleven out of eighteen) with metastasis to the liver, there was definite retention of phenoltetrachlorophthalein even in the absence of bile retention, as shown by the values for the serum bilirubin (Table 2). The degree of dye retention was unquestioned, although less striking than that observed in the jaundiced cases. The maximal reading in the one-hour specimen was 28 per cent. A questionable degree of dye retention was observed in two. However, a normal response was obtained in five of the cases.

The determination of the minimal degree of dye retention that is of clinical significance is important in evaluating the phenoltetrachlorophthalein test in a study such as this. Rosenthal gives the normal readings for phenoltetrachlorophthalein as 7 per cent at fifteen minutes, 3 per cent at one hour, and none at two hours. These represent maximal figures in normal persons. There is considerable variation in hospital cases, and numerous cases are seen in which the readings are slightly higher without there being definite clinical evidence of hepatic disease. We believe that readings of at least 10 per cent at fifteen minutes, 5 per cent at one hour, and 3 per cent at two hours, are required before they can be considered significant of hepatic disturbance. Lesser degrees of dye retention are of questionable significance.

Of the seventeen cases of carcinoma without demonstrable metastasis to the liver, three showed slight dye retention and two others showed questionable retention, in the other twelve elimination curves were normal. In none of these cases did the urine specimen contain more than 1 mg of phenoltetrachlorophthalein without there being at the same time definite retention of dye in the blood stream.

COMMENT

In order properly to evaluate the clinical usefulness of laboratory tests in the demonstration of carcinomatous invasion of the liver, a clear understanding is necessary both of the physiologic activity of the liver and of the pathologic processes concerned in the spread of a malignant tumor. Carcinoma, in general, spreads along lymphatic channels, first involving the adjacent lymph nodes. These may be so situated as to permit of complete surgical removal, although this is often impossible. The liver may be affected by direct extension, as from carcinoma of

the gallbladder or stomach, or through lymphatic or vascular channels. The secondary lesions may be single or multiple, and may vary in size from microscopic aggregations of growing tumor cells to huge masses that replace or destroy the normal structure of the organ. In the one instance the most careful pathologic examination may be necessary to find evidence of the invasion of the liver, while in another the clinical diagnosis may be unmistakable.

Many observers have emphasized the large functional reserve or margin of safety of the liver. Experimentally, we have found that when chloroform is injected intraportally, according to the method of Schultz, Hall and Baker,⁹ it produces multiple areas of necrosis in the liver without affecting the remainder of the organ. In such animals, tests may show normal function. On the other hand, prolonged chloroform anesthesia or the intramuscular injection of chloroform produces uniform parenchymal changes throughout the liver. Marked functional disturbances may readily be demonstrated in these animals.

Similar factors may undoubtedly determine the effect of malignant invasion of the liver. Apart from obstruction to the biliary passages and the production of jaundice, the degree of damage produced by the malignant process will depend, more or less quantitatively, on the extent of the destruction of hepatic tissue. Until there is some infringement of the normal reserve, functional disturbance cannot be expected.

Robertson¹⁰ has emphasized the possibility that tumors in the liver, perhaps through interference with the vascular supply, may produce degenerative changes in the parenchymal tissue over an area quite disproportionate to the size of the metastatic nodule. It is apparent, therefore, that while a normal response to a laboratory test will not wholly exclude the possibility of malignant disease, a positive test may be of great value in indicating the presence of hepatic involvement.

In the first group of cases (carcinoma with manifest jaundice), the functional changes were characteristic and definite. These changes are identical with those observed in the cases of obstructive jaundice previously reported. No distinction is possible on the basis of the laboratory tests between cases of obstructive jaundice due to carcinoma of the head of the pancreas and the present cases of carcinomatous involvement of the liver with jaundice. The differential diagnosis may be extremely difficult in cases of complete biliary obstruction. As in Cases 3 and 5 (Table 1) fluctuations in the degree of jaundice from time to time and the intermittent appearance of bile in the stools do not wholly exclude the possibility of carcinoma, although they are more

9 Schultz, E. W., Hall, E. M., and Baker, H. V. Repair of the Liver Following the Injection of Chloroform into the Portal System, *J. M. Res.* 44: 207-230 (Dec.) 1923.

10 Robertson, H. E. Personal communication to the author.

frequently associated with obstruction due to stone. The effects of the bile retention dominate the laboratory findings, and while the evidence of hepatic disturbance is definite, the tests do not distinguish between the different etiologic agents possibly concerned. The laboratory tests are of value, however, in furnishing quantitative evidence of the extent of the changes produced by the jaundice. The serum bilirubin is of particular value in that it furnishes a much more sensitive and accurate index to the degree of bile retention than does any clinical impression regarding the color of the skin or conjunctivae. Particularly when the obstruction to the biliary passages is of neoplastic origin may the clinical evidences of malignancy be lacking. In the absence of such evidence,

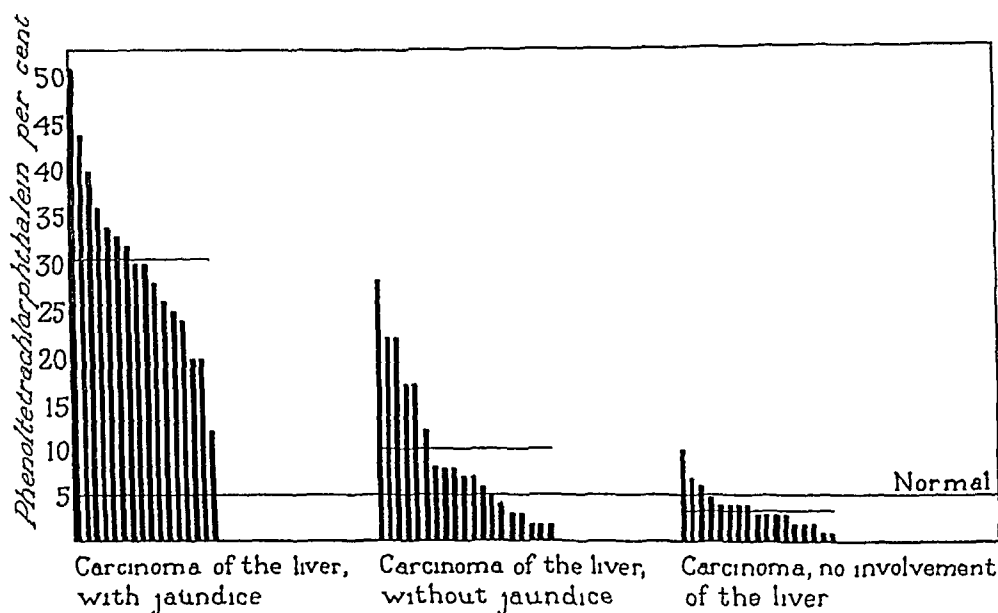


Fig 2—Variations in the degree of phenoltetrachlorophthalein retention in the different types of carcinoma studied. The maximal value level is indicated, and in each group the average value is shown by the horizontal line.

the exact diagnosis is obtained only at operation or necropsy. It is in just such cases that some reliable test indicating the presence of carcinoma somewhere within the body would be of inestimable value.

In the absence of jaundice, the clinical diagnosis of invasion of the liver by a malignant process is largely dependent on the demonstration of enlargement of the liver or of nodules over its surface. These signs are often lacking and the clinical diagnosis is impossible. In the majority of the cases of this type, as shown in Table 2, the phenoltetrachlorophthalein test shows moderate retention of the dye, which we believe to be especially significant. In the presence of clinical evidence of hepatic involvement it is pathognomonic. If the liver is not palpable and carcinoma can be demonstrated elsewhere, the possibility of hepatic involvement must be seriously considered. In a few of these cases,

an increase in the serum bilirubin or latent jaundice was demonstrated. This finding affords valuable confirmatory evidence of the hepatic involvement shown by the phenoltetrachlorophthalein test. As we have pointed out, a negative test does not exclude hepatic damage, but in several of the cases the phenoltetrachlorophthalein test furnished the only evidence of metastasis to the liver. It is in such cases that functional tests assume their greatest significance, since they affect clinical judgment in regard to diagnosis, prognosis and treatment.

Normal responses to the different tests were obtained in practically all of the cases of carcinoma without apparent involvement of the liver. One patient with a history of jaundice following chloroform anesthesia showed slight dye retention, this serves to emphasize the necessity of excluding other forms of hepatic injury before concluding that positive tests truly indicate malignant disease of the liver. Similarly, toxic influences may explain the results in the other three cases in which there was slight delay in the removal of phenoltetrachlorophthalein from the blood stream. It is also possible that in these cases the dye retention was evidence of metastatic involvement of the liver that was not visible to the surgeon. If so, this would further emphasize the value of the phenoltetrachlorophthalein test.

CONCLUSIONS

The determination of the serum bilirubin and the phenoltetrachlorophthalein test promise to be of very definite assistance in the study of patients with abdominal carcinoma and suspected malignant disease of the liver, although the greater number of the other tests for hepatic function studied fail to show significant changes or sufficiently specific changes to be of any great clinical value.

When extension of the malignant process to the liver is accompanied by jaundice, as from obstruction of the extrahepatic bile passages, it is direct evidence of a disturbance in the normal physiologic activity of the liver. In such cases functional tests and the determination of the serum bilirubin are of value in that they furnish a sensitive and quantitative means of studying the effects of the jaundice. The tests are of less value in differential diagnosis, for the jaundice dominates both the clinical picture and the laboratory findings. The jaundice associated with malignant disease in the liver is not to be differentiated by these tests from obstructive jaundice from any cause.

It is in the study of patients with carcinoma involving the liver without jaundice that functional tests are of greatest value, particularly the phenoltetrachlorophthalein test. In the cases in which there was clinical evidence of malignant disease of the liver, confirmatory results were obtained by functional studies. In the absence of clinical evidence of hepatic involvement, the phenoltetrachlorophthalein test may furnish

the only evidence of the existence of metastatic nodules in the liver. Positive tests are not obtained in all cases of metastatic involvement of the liver, for positive tests are a measure of the interference with the activity of the liver as a whole, and the latter is an organ with a large margin of safety. For this reason, however, positive tests when obtained become doubly significant.

There are definite limitations to the use of functional tests in the study of hepatic disturbances, and in our experience the extreme difficulty attending the early clinical recognition of malignant involvement of the liver and biliary passage was again manifest. We believe that the serum bilirubin and the phenoltetrachlorophthalein tests are of clinical value in the early diagnosis of carcinoma of the liver and biliary tract.

NUTRITIONAL CHANGES IN EXOPHTHALMIC GOITER

THE EFFECT OF LUGOL'S SOLUTION [†]

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AND

JAMES A GREENE, M D

BOSTON

The body weight is undoubtedly one of our most valuable indexes of nutrition and indicates clearly, when determined over a long period, the balance between the energy supplied to the body in the form of food and the total energy expended as heat and muscular movements. As exophthalmic goiter is one of the few conditions in which the basal metabolism is characteristically elevated, the relation between the food intake, energy expenditure and body weight in this disease should be of considerable interest. It is well known that every normal individual expends a definite amount of energy depending on sex, age, height, weight, food intake and muscular activity. To provide for this an equal number of calories in the form of food must be ingested or the individual will consume his own tissues and a loss in body weight will follow. If a quantity of food in excess of the energy requirement is eaten, the surplus calories will be stored in the body chiefly in the form of fat and carbohydrate, and there will therefore be a gain in body weight. In a normal person, a diet that is inadequate in calories first results in a loss of body weight. Following this there is a drop in the basal metabolism or energy requirement. In other words a normal person, within certain limits, can adjust his caloric production to meet his caloric intake in the form of food. For example, Benedict ¹ reports a group of normal young men who consumed from 3,200 to 3,600 calories daily while leading an active life. Their diet was reduced until the body weight loss averaged 12 per cent. At this level their basal energy requirement had diminished 18 per cent and these men could then maintain their body weight on an average of 1,950 calories daily. Joslin ² has calculated that, in normal persons, for each 1 per cent loss in weight there is a percentage decrease in the metabolism of 1.8. The fall in the basal metabolism with the undernutrition may be regarded as an attempt to prevent further loss of weight by lowering the total energy expenditure.

[†] From the medical clinic of the Peter Bent Brigham Hospital

1 Benedict, F G. Metabolism During Starvation and Undernutrition, New York M J **115** 249 (March) 1922

2 Joslin, E P. Diabetic Metabolism with High and Low Diets, Carnegie Institution of Washington Publication No 323, 1923, p 72

The basal energy requirement, that is, the amount of energy expended by a person at complete rest in bed is easily calculated from the Aub and DuBois³ standards. For example, a man, aged 35, height 176 cm and weight 75 kg, requires approximately 1,800 calories. To this should be added 10 per cent, or 180 calories, for the specific dynamic action of food. If the person is up and about and pursuing an occupation that involves a moderate amount of muscular exertion, an addition of 50 per cent of his basal caloric requirement should be added. The total number of calories required by such a person per twenty-four hours is, therefore, 2,880. This coincides roughly with numerous observations on the amount of food eaten by normal persons under these conditions.

The foregoing considerations are preliminary to a discussion of the metabolism or energy requirement in exophthalmic goiter and its relation to the food consumption and body weight in this condition. If we consider the example previously cited of a man, aged 35, height 176 cm and weight 75 kg, in whom the normal basal requirement is 1,800 calories per twenty-four hours, and in addition assume the basal metabolism to be 50 per cent above normal, which is a fair estimate in a moderately severe instance of exophthalmic goiter, it is possible to calculate the dietary requirements of such a patient. Following an extended study⁴ of the food consumption and basal metabolism in a patient with exophthalmic goiter, in this hospital in 1922, it was concluded that a caloric intake averaging 84 per cent greater than the basal metabolism was necessary in order to maintain weight. Boothby and Sandiford⁵ have made extensive studies of the total metabolism in nine patients with exophthalmic goiter and give in a detailed table, among other information, the basal metabolism and food intake per twenty-four hours for each patient before surgical measures were instituted. These subjects were at complete rest in bed or up from two to three hours daily. The average intake was 3,517 calories per twenty-four hours, or 78 calories per kilogram of body weight. Each patient remained in a positive nitrogen balance and there was a very slight gain in weight, averaging 75 gm per twenty-four hours for the group. In order to accomplish this, it was necessary for each patient to consume an average of 3,517 calories daily, which was an intake 90

3 Aub, J. C., and DuBois, E. F. Clinical Calorimetry, XIX, The Basal Metabolism of Old Men, *Arch Int Med* **19** 823 (May) 1917.

4 Sturgis, C. C. Observations on One Hundred and Ninety-Two Consecutive Days of the Basal Metabolism, Food Intake, Pulse Rate and Body Weight in a Patient With Exophthalmic Goiter, *Arch Int Med* **32** 50 (July) 1923.

5 Boothby, W. M., and Sandiford, Irene. The Total and Nitrogenous Metabolism in Exophthalmic Goiter, *J A M A* **81** 795 (Sept 8) 1923.

per cent greater than the average basal metabolism of the group. For the maintenance of body weight it seems reasonable to conclude that, in addition to the extra energy required by the increase in the basal metabolism, it is necessary to add another increment of from 75 to 100 per cent of the basal metabolism on account of the specific dynamic action of the food eaten and the excessive activity of these patients even though they are resting in bed most of the time. If the energy requirements are calculated under such conditions, we should add 900 calories to the normal basal requirement of 1,800 calories of the person previously considered for the increase of 50 per cent in the basal metabolism, and 85 per cent of the elevated basal, or 2,295 calories, as an average for the muscular movements of a patient in bed and the specific dynamic action of food. The total amount, therefore, is found to equal 4,995 calories. Such an estimate indicates the number of calories necessary to furnish a patient of this age, height and weight, having a basal metabolism 50 per cent above normal, with sufficient food in order to maintain body weight while resting in bed. It would be difficult to estimate, with any degree of accuracy, the dietary requirement necessary if such a person were carrying on an occupation requiring moderate or heavy exertion. Boothby and Plummer⁶ have reported that in hyperthyroidism a given amount of work requires almost twice the energy expenditure that it does in normal persons, or, in other words, such a patient is very inefficient in the performance of mechanical work, which is a matter of considerable importance in estimating their total energy expenditure. A conservative estimate of the caloric requirements under these circumstances would be in the vicinity of 6,000 calories or more, an amount that represents a very large quantity of food and requires an unusually good appetite before it can be ingested.

The various factors governing the caloric requirement in normal persons who are undernourished are not effectual in patients with exophthalmic goiter. While undernutrition in an otherwise normal person is followed by a fall in the basal metabolism, the same is not true in a patient with exophthalmic goiter, for in this condition, although many pounds of body weight may be lost, the constant stimulus to cellular metabolism characteristic of the disease elevates the basal metabolism to a level that may reach 75 or 100 per cent above normal. Therefore, although the food intake may be diminished, the basal metabolism or energy expenditure remains at a level often greatly above normal, and as a result the patient's own body tissues are burned to such an extent that a striking loss of body weight results unless a very large quantity of food is eaten.

⁶ Plummer, H. S., and Boothby, W. M. The Cost of Work in Exophthalmic Goiter, *Am. J. Physiol.* **63**: 406, 1922-1923.

DuBois⁷ has emphasized that the normal subject's appetite unconsciously balances the bodily needs and maintains a practically constant weight in many people with extraordinary exactness. If a large percentage of normal persons meet their energy requirements by consuming an approximately exact quantity of food, the question may be asked how adequately are the increased energy requirements met in a patient with exophthalmic goiter and an increased metabolism.

In order to secure information on this question, the histories of sixty-five patients have been considered in order to ascertain the amount of weight lost and to gain an approximate idea of the food intake from their own statements concerning their appetites over the duration of the disease. Sufficient data was obtainable on which an opinion might be based in fifty-two of the sixty-five histories. This information revealed that in 86.5 per cent, or 45 patients, of the 52, there was a loss of body weight averaging 11.9 kg (26.3 pounds) in an average length of time of 11.9 months. Six patients stated that there had been no variation in body weight, and in one it was claimed that the weight had increased 11 pounds (5 kg). When we consider the rather striking weight loss in such a high percentage of these patients over a relatively short interval, it is interesting to obtain an estimate of their food intake. The patient's appetite was classified as ravenous, increased, normal or decreased. In eighteen, or 34.6 per cent, the appetite was considered as ravenous, in eleven, or 21.2 per cent, as increased, in seventeen, or 32.7 per cent, as normal, and in six, or 11.5 per cent, as decreased. In summary it can be said that in a total of forty-six patients, or 88.5 per cent, the appetite was regarded as ravenous, increased or normal. When it is considered that 86.5 per cent of the same fifty-two patients lost an average of 11.9 kg (26.3 pounds) in 11.9 months, it emphasizes in a very striking manner the inability of most patients with exophthalmic goiter, when leading a more or less normal life, to consume a diet adequate in calories to provide for the greatly augmented energy expenditure. In a small number of patients with exophthalmic goiter having a basal metabolism elevated above normal limits, however, it is apparently possible to ingest sufficient food to maintain body weight, and even in rare instances to increase it, despite the large energy requirements. Under the latter circumstances the appetite must be extraordinarily large. If the patient's food consumption is persistently small over a long interval and yet his activities are not limited, the loss of weight is often remarkable, resembling the decrease frequently observed in severe untreated diabetes. In the last analysis, variation in body weight in patients with exophthalmic goiter is dependent on the balance

⁷ DuBois, Eugene. *Basal Metabolism in Health and Disease*, Ed 1, Philadelphia, Lea and Febiger, 1924, p 181

between the amount of energy which is available to the body in the form of food and the total energy expended, which depends chiefly on the amount of muscular exertion and the level of the basal metabolism. From a consideration of the available data it is apparent that most patients, although they have a large appetite, cannot supply their increased energy requirements and therefore lose weight, while a very small percentage can ingest sufficient food to maintain and even, in rare instances, to gain weight.

The rather unusual history of a weight loss and a good or excessive appetite is not frequently encountered in clinical medicine and is information of a particularly definite nature which suggests the possibility of an increased metabolism, it is therefore of value from a diagnostic standpoint. Under ordinary circumstances undernutrition, as it is usually observed in clinical medicine, is associated with a poor appetite and a diminished food intake. The striking exceptions to this are found in diabetes and exophthalmic goiter. In these conditions similar degrees of undernutrition are frequently observed. Joslin⁸ found, in a study of 200 patients with diabetes, that the average weight loss was 12 kg (26.4 pounds), which represented the difference between the maximum weight before the onset of the disease and the weight when the patient was first observed. In the group of patients with exophthalmic goiter who lost weight (86.5 per cent of the group), the decrease was almost precisely the same, as the average loss was 11.9 kg (26.3 pounds). Joslin⁹ reports that the respiratory quotients of the severe cases of diabetes studied at the Nutrition Laboratory between 1908 and 1911 averaged 0.73, which indicated that very small amounts of carbohydrate were being utilized. It is interesting to note that the respiratory quotients of this group of patients with exophthalmic goiter averaged approximately the same, the figure being 0.75. Although in individual instances no great importance can be attached to small changes in the respiratory quotient, they are of considerable significance when the quotients of a large group of patients are considered. Quotients of 0.73 and 0.75 in these two groups of patients indicate that 8.4 per cent and 15.6 per cent, respectively, of the total calories are derived from carbohydrate, whereas the average quotient of 0.83, which was found in 236 normal persons in the Harris and Benedict¹⁰ series, means that 43.8 per cent of the calories produced were due to the combustion of carbohydrate. In severe, uncomplicated diabetes a low respiratory quotient

8 Joslin, E. P. The Prevention of Diabetes Mellitus, J. A. M. A. 76:79 (Jan. 8) 1921.

9 Joslin, E. P. (Footnote 2, p. 165).

10 Harris and Benedict. Carnegie Institution of Washington Publication No. 279, 1919, pp. 40 and 44, Tables C and D, quoted by Joslin (Footnote 2, p. 167).

is expected as in a subject with this disease there is an inability to utilize carbohydrate. In exophthalmic goiter there is no impairment of the ability to burn carbohydrate but owing to the increased energy requirements of the disease, the glycogen stores are constantly called on and are therefore greatly depleted, which is further evidence of undernutrition in this condition.

COMPARISON OF BODY WEIGHTS OF PATIENTS WITH EXOPHTHALMIC GOITER WITH STANDARD WEIGHT TABLE
BASED ON HEIGHT, AGE AND SEX

To secure further information concerning the nutrition of patients with exophthalmic goiter, the body weights of sixty-five such patients were compared with the Medico-Actuarial Weight Tables,¹¹ which have been utilized by Joslin⁸ in a study of the body weight of patients with diabetes. It was impossible to use the more recent weight standards proposed by Dreyer and Hanson¹² which are based on stem length and chest girth, as these measurements were not available in this group of patients. The comparison of the actual body weights of the sixty-five patients with the weight tables showed that twelve, or 18 per cent, averaged 68 per cent greater than is considered normal according to the standards used. One patient of this group appeared obese and was actually 15.9 kg (35 pounds) over her estimated normal. One patient of the 65, or 1.5 per cent, had a body weight that coincided exactly with the calculated normal. In fifty-two of the sixty-five patients, or 80 per cent, the body weight was found to average 18.2 per cent below the normal. Judging from clinical experience and from these observations it seems safe to conclude that the body weight of about 20 per cent of all patients with exophthalmic goiter will be normal or slightly above and that they will appear well nourished, in rare instances, they may even be slightly obese. The remaining 80 per cent will give evidences of loss of body weight, which will average, if a group is considered collectively, about 18 per cent of their body weight before the onset of the disease.

In making these conclusions, the question quite properly arises of the reliability of the normal weight tables which were used. It is, of course, impossible to estimate precisely the ideal weight of a person basing the calculations on height, age and sex. The normal standards that were utilized are considered among the most reliable available. An

11 Medico-Actuarial Mortality Investigation, Association of Life Ins. Med. Directors and Actuarial Soc. of America, New York 1:37 (Table 3), 66 (Table 8), 1921.

12 Dreyer, Georges, and Hanson, G. F. The Assessment of Physical Fitness, New York, Paul B. Hoeber, 1921.

individual variation of 10 per cent below normal cannot be considered significant, but if a group of sufficient size is considered collectively and their average is found to be 10 per cent below the normal, it indicates a definite abnormal variation¹³ In order to ascertain the accuracy of these normal standards we have compared the actual body weights of a group of normal persons, male and female, with the normal weights given by the tables In thirty-five males between the ages of 19 and 54 years, the actual body weight averaged 2.9 per cent below the weights given in the standard tables In thirty-five females, between the ages of 20 and 53 years, the variations between the actual were 3 per cent below the calculated body weight The average variation from the normal standard of the entire group of seventy people was 2.97 per cent below the calculated body weight These deviations from the normal standard seem surprisingly small and appear to justify the conclusions concerning the body weight of patients with hyperthyroidism which have been given above Additional data is available furthermore which indicates the accuracy of these standards when applied to groups of persons Our conclusion has been that 80 per cent of the group of patients with exophthalmic goiter have a body weight almost 20 per cent below the normal standard when they present themselves at this hospital for examination The question may be asked, How many normal persons will be 20 per cent below these standards? This may be answered by a consideration of the facts shown by the New England Mutual Life Insurance Company Mortality Investigation between the years 1844 and 1905, when it was found that of the 136,240 insured healthy persons only 4.6 per cent were 20 per cent underweight⁸ according to these standards These comparisons therefore indicate that considerable significance can be attached to the statistics concerning body weight in patients with exophthalmic goiter when a group of sufficient size is considered

It is apparent, then, that a large majority (80 per cent) of these patients, when they appear at this hospital for treatment after having had exophthalmic goiter for an average interval of 16.1 months, show rather striking undernutrition This results from an inability to provide by their diet for the high caloric requirement due to a characteristically high basal metabolic rate, an inefficiency in the performance of mechanical movements, and a loss of the normal adjustment mechanism which makes possible a drop in the basal metabolism as undernutrition develops When the dietary requirements are inadequately met, the patient's reserve stores of fat and carbohydrate are drawn on as sources of energy Ultimately if these fail to provide sufficient energy, body pro-

¹³ Root, H. F., and Miles, W. R. *Physical Measurements of Diabetic Patients*, *J. Metabolic Res.* 2: 190 (Aug.) 1922

tein is consumed as food, and under these circumstances a loss of nitrogen results. According to our figures such a patient may be represented theoretically by a person whose weight has decreased from a normal of 68.2 kg (150 pounds) to 58 kg (123 pounds), a loss of 18.2 per cent, throughout an illness of 16.1 months. During this interval it is reasonable to suppose that periods of nitrogen loss may be present owing to an inadequate diet. Such a patient's condition, with reference to undernutrition, is somewhat comparable to that of a subject who has undergone a complete fast for twenty-nine days and as a result suffers a loss in body weight of 20.1 per cent, which is the average loss in that length of time as indicated by Benedict¹⁴ in his resumé of fasting. This amount is similar to the 18.2 per cent loss which occurs in many patients with exophthalmic goiter, although in the latter the decrease in weight is distributed over a much longer period. The foregoing summary emphasizes the magnitude of the loss in body weight which frequently occurs in patients with this disease. More important still, it is such an undernourished patient who comes to a hospital and in a variable period of time, often too brief, undergoes a major operation on the thyroid gland.

LOSS OF BODY WEIGHT IN PATIENTS WITH EXOPHTHALMIC
GOITER FOLLOWING MAJOR OPERATIONS ON
THE THYROID GLAND

The importance of an additional loss of weight following operative procedures in an already undernourished patient led to a careful survey of the percentage loss of weight in these patients following partial or subtotal thyroidectomy. The question of determining the change in body weight and analyzing accurately the factors responsible for this change is not as simple as it may appear on first inspection. In studying these patients the preoperative body weight, without clothes, was recorded at the time of the last determination of the basal metabolism, which was usually within two or three days before operation. The patient was weighed, usually between ten and eleven days after the operation, when the first metabolism following the operation was determined. This weight loss recorded ten or eleven days after the operation probably does not indicate accurately the maximum as by this time the postoperative febrile reaction had disappeared, food could be taken in fair amounts, and the metabolism was usually normal, according to the observations of Segall and Means¹⁵. It seems a reasonable estimate to

14 Benedict, F. G. A Study of Prolonged Fasting, Carnegie Institute of Washington Publication No. 203, 1915, p. 80.

15 Segall, H. N., and Means, J. H. The Immediate Effect of Subtotal Thyroidectomy in Toxic Goiter, Daily Basal Metabolism and Pulse Observations, Arch. Surg. 8: 176 (Jan.) 1924.

assume that the maximum loss of body weight occurs from the fourth to the sixth postoperative day, and that several pounds may be gained between that time and the day on which the patient is actually weighed. The error resulting from weighing the patient on the tenth or eleventh postoperative day would therefore tend to minimize the loss of weight following operation.

TABLE 1—*Percentage Variation in Body Weight Following Subtotal Thyroidectomy in Thirty-Six Patients with Exophthalmic Goiter Who Were Not Treated with Lugol's Solution Prior to Operation*

Case	Variation in Body Weight Following Operation, per Cent *	Postoperative Day Weight Was Determined	Metabolism Before Operation	Metabolism After Operation†
1	-6.4	18	+29	-3
2	-5.3	11	+43	+18
3	-6.2	3	+33	+75
4	-2.9	11	+37	+16
5	-7.7	6	+41	+16
6	-9.7	8	+38	+11
7	-6.8	14	+29	+17
8	-3.5	11	+28	-9
9	-6.9	6	+63	+42
10	-4.0	11	+21	+8
11	-5.3	17	†	†
12	-1.5	11	†	†
13	-2.4	18	†	†
14	-5.0	10	†	†
15	-3.1	12	†	†
16	-5.5	14	†	†
17	-3.8	7	+54	+30
18	-5.2	10	+33	+14
19	-6.4	11	+34	+12
20	-4.3	10	+26	+39
21	-4.2	13	+44	+40
22	-5.4	8	+37	+7
23	-6.7	10	+31	-3
24	-3.5	10	+13	-5
25	-5.6	7	+40	
26	-5.9	16	+50	+24
27	-1.0	15	+29	+10
28	-7.9	12	+34	+24
29	-5.6	5	+17	-4
30	-4.6	12	+44	+21
31	-11.9	15	+49	+28
32	-12.5	13	+71	+29
33	-2.9	10	+55	+33
34	-4.1	11	-39	+3
35	-6.0	15	-26	+12
36	±0.0	9	+53	+36
Average	-5.2	11	+39	+18

* The minus sign indicates a loss of weight.

† The metabolism determination was made on the same day the postoperative body weight was obtained, as indicated in the second column.

‡ The basal metabolism was not determined in these patients.

The loss of body weight following a major operation on the thyroid gland in thirty-six consecutive patients with exophthalmic goiter is shown in Table 1. It is to be emphasized that every single patient lost in body weight except one, in whom it was unchanged. The greatest loss was 12.5 per cent, while the smallest was 1 per cent. The average loss was 5.2 per cent in an average of eleven days, which would represent, for example, a decrease of 3.5 kg (7.7 pounds) in a patient whose original body weight was 68.2 kg (150 pounds) immediately before the

operation This weight loss assumes more importance when one considers that these patients are usually undernourished before the operation and suffer an additional weight loss of considerable extent in a few days following the operation It is possible to illustrate the changes in nutrition more strikingly if the percentage changes are applied to actual body weights For instance, if a patient weighed 68.2 kg (150 pounds) before the onset of the disease and then lost 12.9 kg (26.3 pounds), which was the average amount lost in 86.5 per cent of these patients according to their own statements, the weight on admission to the hospital would be 56.1 kg (123 pounds) Eleven days after operation, with an average loss of 5.2 per cent, or 2.9 kg (6.4 pounds), the patient's weight would be 53.3 kg (117.3 pounds) Therefore, the total loss of weight estimated to occur in a patient with exophthalmic goiter whose original weight before the onset of the disease was 68.2 kg (150 pounds) and who fell into the group of undernourished patients, would be 14.9 kg (32.7 pounds), or 21.8 per cent, including the decrease in weight following the operation

ANALYSIS OF FACTORS RESPONSIBLE FOR LOSS OF WEIGHT
IN PATIENTS WITH EXOPHTHALMIC GOITER
FOLLOWING OPERATION

Among the important factors which must be considered and which may be responsible for fluctuations in body weight following operation may be mentioned the level of the basal metabolism immediately before and after the operation, the extent of the postoperative febrile reaction, the ability of the patient to ingest food, which may be influenced by postoperative nausea and vomiting or difficulty in swallowing due to trauma about the neck, the type of anesthetic, postoperative acidosis, and changes in the water balance of the body All of these factors may be of importance in the same patient, but in a consideration of this entire group an attempt has been made to determine the relative importance of each of these

The most rapid fluctuations in body weight are associated with alteration in the water balance of the body Striking illustrations of this are found in the data obtained by Professor William G. Anderson of the Yale University Gymnasium, who observed that a football player lost 6.4 kg (14 pounds) in one hour and ten minutes, a Marathon runner lost 3.9 kg (8.5 pounds) in three hours, and a member of a college eight-oared boat crew lost 2.5 kg (5.5 pounds) in twenty-two minutes¹⁶ The decrease in body weight was due in each instance to increased destruction of body tissue and loss of water The former was

¹⁶ Anderson, W. G., quoted by Benedict, F. G., and Joslin, E. P. *A Study of Metabolism in Severe Diabetes*, Carnegie Institution of Washington Publication No. 176, 1912, p. 96

insignificant as indicated by the calculations of Benedict and Joslin,¹⁶ who estimated that during an hour of strenuous muscular exertion, similar to the exercise of a football game, not more than 100 gm (about one-fourth pound) of dry organic material would undergo combustion in the body. Subtracting this from the total loss of weight, there is left 6.3 kg (13.75 pounds) of lost weight due to water. It is of importance to note that in these persons there was no fluid intake or a very slight one during the intervals of exercise, and that the body weight of the football player returned to normal inside of forty-eight hours following food and drink. While there may have been fever and excessive sweating for a few days during the postoperative febrile period in the patients observed, there is no indication that an excessive quantity of fluid was lost from the body. Immediately after the operation the fluid intake was maintained in the vicinity of 2,500 cc every twenty-four hours by means of subpectoral saline injections and dextrose solution by rectum. In addition, sips of water by mouth were permitted, beginning a few hours after the operation. The only factor of significance concerning the water balance is a possible accumulation of edema due to cardiac failure, which may be intensified as a result of the operation. A few of these patients had some evidence of cardiac failure with moderate edema before the operation, but they were not operated on until the edema had disappeared as a result of rest in bed and digitalis. While there was no visible edema after operation, it is well known that approximately 10 pounds (4.5 kg) fluid may collect in the body tissue without giving definite evidence in the form of "pitting." The collection of edema may introduce an error in the weight change, as it may tend to mask any real loss of weight. In this group of patients, however, there was no indication that a change in the body weight due to the accumulation or loss of edema was of importance.

Previous to the collection of data, it was thought that the effect of the various anesthetics might be related to the magnitude of the weight loss following operation. Apparently the type of anesthetic had no significance in this respect, as shown by the following data, although the number of patients was too small to afford conclusive evidence. Of the thirty-six patients, eighteen were given ether and the average weight loss was 4.9 per cent, eight patients were operated on under procain and the average weight loss was 4.64 per cent, eight were given nitrous oxid and oxygen and had an average weight loss of 6.51 per cent, one had a combination anesthesia of nitrous oxid, oxygen and procain which was followed by a loss in weight of 6.4 per cent, and one had nitrous oxid, oxygen and ether, with a loss in weight of 5.29 per cent. It seems logical to assume that the administration of ether as an anesthetic will result in the most severe postoperative symptoms, which will

be followed by the greatest loss in body weight This is not supported by the foregoing data, nor is there any conclusive evidence for or against any one particular anesthetic as far as the loss of body weight is concerned

Goetsch and Browder¹⁷ have emphasized that acidosis is very prone to occur after operations on the thyroid gland and that acetone may appear in small amounts in the urine even immediately before operation According to these investigators, the postoperative acidosis is responsible for marked restlessness, often distressing nausea and vomiting, headache, irritability of the bowel with diarrhea, a dry tongue, parched lips, acetone odor to the breath and often large amounts of acetone in the urine Mild postoperative acidosis, as evidenced by acetone bodies in the urine, has been observed in a fair number of these patients, although never in any instance has a severe acidosis been encountered It appears difficult to ascertain to what extent the postoperative symptoms are due to the formation of ketone bodies, but it does not seem possible that they can be a factor of the greatest importance in the loss of weight following thyroid operations The development of an acidosis does emphasize the undernutrition of these patients and suggests very strongly that the glycogen reserves of the body in patients with exophthalmic goiter are usually very low Therefore, for several days after the operation, when very little or no food is being consumed, the available body glycogen is completely exhausted and an acidosis may result

The inability to take food owing to nausea, vomiting and difficulty in swallowing as a result of trauma about the neck is undoubtedly of importance and accounts, in part at least, for the loss of some weight following major thyroid operations It is not the sole factor, as indicated by a study of the postoperative loss of weight in a series of twenty-five patients with colloid goiter and nontoxic adenoma, all of whom had either a normal metabolism or no clinical evidence that their basal metabolism was increased Twenty-two of these patients were given ether as an anesthetic Reference to Table 2 shows that the average loss in body weight in these patients averaged only 2.47 per cent as compared to an average loss of 5.2 per cent in patients with exophthalmic goiter and an elevated metabolism As almost all of the patients with colloid goiters and nontoxic adenomas had ether and the operative procedures were at least comparable in extent to that employed in exophthalmic goiter, it seems logical to assume that the postoperative nausea, vomiting and difficulty in taking food following the operation would be similar in the two conditions So, while postoperative complications are

17 Goetsch, E, and Browder, E J Studies on Thyroid Disorders, IV, Intravenous Administration of Glucose Solution in Treatment of Acidosis Following Thyroid Operations, New York State J Med 22:469 (Oct) 1922

of importance in exophthalmic goiter, they explain only approximately one half of the weight loss and it becomes necessary to attribute the remainder to some other factor

It has been previously emphasized that patients with exophthalmic goiter who lose body weight do so because their energy expenditure is at such a high level that their food intake fails to supply an adequate number of calories and the patient consumes his own body tissues. Following subtotal or partial thyroidectomy the food intake is practically nil for a period of several days and it is usually eight or ten days or longer before the diet is taken in normal amount. In the series of thirty-six patients shown in Table 1 the metabolism was elevated in each

TABLE 2—*Percentage Loss of Weight in Twenty-Five Patients with Colloid Goiter and Nontoxic Adenomas*

Case	Variation in Body Weight Following Operation,* per Cent	Postoperative Day Weight Was Determined
1	-2.51	14
2	-1.66	3
3	-0.41	8
4	-3.64	10
5	-2.72	15
6	-5.88	7
7	-3.34	9
8	-3.54	8
9	-1.17	10
10	-2.36	12
11	-3.64	7
12	-5.1	4
13	-2.76	8
14	-2.94	5
15	-6.00	8
16	-3.68	6
17	-2.56	8
18	-4.04	11
19	-2.24	8
20	-1.35	11
21	-4.57	7
22	-3.82	12
23	+3.41	8
24	+1.86	12
25	+2.87	12
Average	-2.47	8.9

* The minus sign indicates a loss, the plus sign a gain in body weight

instance before the operation, the average being 39 per cent above the normal. While the basal metabolism returned to normal within from two to three weeks in almost every instance following the operation, it did remain elevated in a number of patients for a period of ten days, and during the first three or four postoperative days it was probably elevated above the preoperative level, owing to the characteristic febrile reaction after these operations, at which time the body temperature may reach 101 degrees or higher. At this time the highly undesirable combination of a markedly elevated metabolism associated with no food intake, or a very low one, exists in a patient. This is obviously the most important cause of the weight loss following operation in these patients.

That a loss in body weight commensurate with that actually observed could result in a few days in a patient without food and with an elevated basal metabolism is easily verified by calculation. It has been previously estimated that a patient, of average size, having a basal metabolism of 50 per cent above normal would require 4,995 calories to maintain body weight while resting in bed. For several days immediately after the operation it is reasonable to suppose that additional restlessness and the usual postoperative fever would easily raise the caloric requirement to even a higher level. During the first three postoperative days ordinarily a negligible amount of food is ingested, while the minimum energy expenditure for this interval is assumed to be 4,995 calories a day, or 14,985 calories for the total period. Joslin¹⁸ has calculated that each kilogram of body weight lost represents 3,766 calories, having based his figures on the amount of weight lost and the total metabolism in a subject who underwent a total fast of thirty-one days at the Nutrition Laboratory in Boston. Assuming that it is correct to apply this figure to a patient with exophthalmic goiter, it is apparent that a total loss of 3.98 kg (8.7 pounds) would result in three days under these conditions. If the subject's original weight before the operation was 68.2 kg (150 pounds), this would represent a loss of almost 6 per cent, which is in approximate agreement with the average actual recorded loss of 5.2 per cent in the thirty-six patients of Table 1.

Additional evidence that corroborates the suggestion that the high metabolism and small food intake are responsible for the great weight loss is to be found in Table 3, which shows the postoperative decrease in body weight in twenty-eight consecutive patients with exophthalmic goiter who had been treated with Lugol's solution for an interval of from ten to fifteen days prior to the operation. Following the administration of iodine the characteristic drop in metabolism, which resulted in an average basal metabolism of $+21$ for the group just prior to the operation, was obtained.¹⁹ This is in contrast to the group of thirty-six patients who were not treated with Lugol's solution and in whom the average metabolism was $+39$ just before the operation. In the group of twenty-eight patients treated with Lugol's solution, five, or 17.8 per cent, actually weighed more on the tenth postoperative day than they had before the operation, while the average loss of weight for the entire group following operation was 2 per cent, as contrasted to a loss of 5.2 per cent for

18 Joslin (Footnote 2, p. 68)

19 Boothby, W. M., and Plummer, H. S. The Value of Iodine in Exophthalmic Goiter, *J. Iowa M. Soc.* **14** 66 (Feb.) 1923. Starr, Paul, Segall, H. N., and Means, J. H. The Effect of Iodine in Exophthalmic Goiter, *Arch. Int. Med.* **34** 355 (Sept.) 1924. Mason, E. H. The Use of Lugol's Solution in the Treatment of Exophthalmic Goiter, *Canad. M. A. J.* **14** 219 (March) 1924. King, B. T. The Treatment of Exophthalmic Goiter With Iodine, *Northwest Med.* **23** 165 (April) 1924.

the group who were not treated with Lugol's solution and therefore entered the operation with an elevated metabolism. When applied to actual figures these percentage values show that if a patient's body weight before the operation is 68.2 kg (150 pounds) and he is not treated with Lugol's solution, and therefore enters the operation with an elevated metabolism, the loss of body weight will average 5.2 per cent, or 3.5 kg (7.8 pounds), which will result in a body weight of 64.6 kg (142.2 pounds) on the tenth postoperative day. If the patient

TABLE 3—*Percentage Variation in Body Weight Following Subtotal Thyroidectomy in Twenty-Eight Patients with Exophthalmic Goiter Who Had Been Treated with Lugol's Solution Prior to Operation**

Case	Variation in Body Weight Following Operation, per Cent †	Postoperative Day Weight Was Determined	Metabolism Before Operation	Metabolism After Operation‡
1	-1.5	12	+19	-2
2	-2.6	10	+31	+12
3	-3.7	18	+23	+9
4	-3.1	8	+16	+10
5	-1.5	8	+5	+7
6	-2.0	8	+15	+4
7	-1.5	10	+25	+5
8	-4.8	9	+20	+22
9	-2.5	3	+50	+59
10	-0.8	11	+32	+10
11	-0.3	11	+25	+3
12	-0.8	17	+11	+29
13	-7.0	12	+15	+18
14	-3.1	7	+14	-10
15	-1.0	4	+28	± 0
16	-1.6	8	+16	-5
17	-0.7	11	+14	-13
18	-3.8	9	+24	+11
19	-3.5	6	+26	+7
20	-3.9	18	+4	+17
21	-3.6	7	+10	+1
22	-6.3	14	+28	+16
23	-8.2	5	+25	+34
24	+8.7	11	+28	-7
25	+0.4	10	+20	-11
26	+0.6	10	+15	-17
27	+2.5	9	+13	+13
28	+0.9	9	+35	+14
Average	-2.0	9.8	+21	+8.4

* The initial metabolism of this group averaged +55 on admission to the hospital and before Lugol's solution was given. This is approximately the same as the average initial metabolism of the group shown in Table 2.

† The minus sign indicates a loss of weight.

‡ The metabolism determination was made on the same day the postoperative body weight was obtained, as indicated in the second column.

is treated with Lugol's solution, the calculated weight loss will be 2 per cent on the tenth postoperative day and the patient's body weight will be 66.8 kg (147 pounds), which is a significant difference from 64.6 kg (142.2 pounds) as indicated above.

The practical suggestions that may be deduced from these observations are obvious. If a patient falls into the undernourished group as indicated by a comparison of the actual with the normal weight tables, and in addition gives a history of a fairly large loss of weight, treatment should first be directed toward the undernutrition, a phase of the dis-

ease that is, frequently ignored. If the treatment is to be a subtotal thyroidectomy, then the patient should be under observation for a variable period, depending on the degree of undernutrition. If the body nutrition is good and there has been no loss of weight or only a small amount, then a period of rest in the hospital of only ten days or two weeks is usually sufficient. At this time Lugol's solution should be administered, and after the third or fourth day the basal metabolism should be determined daily in order to ascertain its lowest level, at which time the subtotal thyroidectomy should be performed. Previously it had been our custom to precede the major operation with one or more preliminary ligations of the thyroid arteries. As the preoperative administration of Lugol's solution has come into greater use, these ligations have been eliminated, with no untoward results. The preliminary period of rest in bed also permits careful study of the heart and the institution of proper therapy when indicated. A patient who is markedly undernourished presents a problem of considerable importance. Such a patient should be under observation resting in bed for a period of four or five weeks or longer. During this time every attempt should be made to give a diet averaging between 75 and 100 per cent greater than the basal metabolism per twenty-four hours. At the same time an effort should be made to decrease the patient's caloric requirements by enforcing strict rest in bed. The management of the diet in patients with exophthalmic goiter requires the greatest skill and resourcefulness. The production of nausea by using foods too sweet or too high in fat content should be avoided. The only food which some foreign patients desire is often of a special character and is obtained with difficulty. Frequently the patient who will not eat can be made to eat by the simple procedure of carefully investigating the character of the articles of diet desired and supplying food that is relished. When possible, charting of the caloric intake is worth while, as it furnishes an accurate index of the food consumed and is an added incentive to the patients, the nurses and attending physicians to keep the caloric intake at a high level.

It is usually not desirable to begin Lugol's solution in these undernourished patients until the probable time of operation is known, unless the patient's condition is serious and demands immediate attention. Otherwise, the optimal effect will probably occur from the iodine therapy in ten or twelve days, at which time the gain in weight may not have been as much as desired. If a further interval is required in order to gain weight, the possibility that the metabolism might gradually rise, despite the administration of iodine, must be considered under these circumstances. The most rational procedure is to keep the patient at complete rest in bed for several weeks, forcing him to take as much food as possible. If the weight curve at that time is upward and the

patient had made a substantial gain, then Lugol's solution should be given for an average of about ten days, in order to reduce the metabolism to the lowest level in preparation for operation

The postoperative treatment of such patients should be followed with the greatest care. Lugol's solution should, of course, be continued on the day of the operation, by rectum if necessary, and for several weeks following the operation. In addition every attempt should be made to supply an adequate fluid intake. This may be accomplished by giving sips of water or other liquids within a few hours after the operation, provided nausea and vomiting are not contraindications. If necessary 1,500 c c of physiologic sodium chlorid solution may be given subpectorally in twenty-four hours. It is also desirable to provide as much food as can be taken immediately following the operation. This may be accomplished by giving orange juice and other drinks containing carbohydrates at the earliest possible moment, due care being taken that nausea and vomiting are not induced. It is of decided advantage, furthermore, to give as many calories as possible by rectum, although it has been said that only from 25 to 35 per cent of nourishment necessary to maintain nitrogenous equilibrium and weight can be given in this manner²⁰. Rectal feeding is best accomplished by giving 1,000 c c of water containing 50 gm of dextrose and 50 gm of ethyl alcohol in doses of 250 c c from every four to six hours. Although the caloric content of this solution is not great (550 calories per thousand cubic centimeters of solution) it is the optimal mixture from a caloric content that is non-irritating and can be retained and absorbed by the patient. Solid food and a general well balanced high caloric diet should be taken by the patient as soon as possible during convalescence. The combination of these simple measures, in an attempt to supply adequate fluid and as much nutriment as possible, and the reduction in the metabolism accomplished by Lugol's solution diminish the tendency of the patients to lose weight following operation. The postoperative loss of weight is of great importance, as the patients are already undernourished, and the measures to conserve weight at this time add safety to the operation and decrease the length of convalescence.

SUMMARY

The relation between the food intake, basal metabolism and body weight in patients with exophthalmic goiter has been emphasized. From our present observations, which are in accord with those made previously, it is concluded that in order to maintain body weight while resting in bed the food intake in a patient with exophthalmic goiter and

²⁰ Carter, H. S. *Artificial Methods of Feeding in Endocrinology and Metabolism*, New York, D. Appleton & Co. 1922, 3: 810

an elevated metabolism must be at least from 75 to 100 per cent greater than the basal metabolism. A study of the histories of sixty-five patients with exophthalmic goiter indicated that although 88.5 per cent of them had an appetite which was classified as ravenous, increased or normal, yet 86.5 per cent of these patients gave a history of a loss of 11.9 kg (26.3 pounds) of body weight in 11.9 months. A comparison of the body weight of these sixty-five patients with the standard weight tables shows that 80.2 per cent averaged 18.2 per cent below normal when they first appeared at the hospital. A study of the alteration in body weight following operation in thirty-six patients who had not been treated with Lugol's solution and therefore were operated on with an elevated metabolism, averaging $+39$, showed that all except one, in whom the weight was unchanged, lost an average of 5.2 per cent of their preoperative body weight in an average of eleven days after the operation. The factors responsible for this loss of weight might be several, but it was concluded that the most important was the combination of the inability of the patient to consume normal quantities of food and the elevated metabolism, which may remain high for a period of ten days or longer following thyroidectomy. A second group of twenty-eight patients, who had been treated with Lugol's solution and in whom the metabolism was reduced to an average of $+21$ before the operation, was studied. In these patients, 18 per cent had actually gained weight by the tenth postoperative day, and the loss of weight for the entire group averaged only 2 per cent. This loss is even less than the average decrease in body weight following operation in a group of twenty-five patients with colloid goiters and nontoxic adenomas.

GLYCEMIA AS A GUIDE IN THE TREATMENT OF DIABETES MELLITUS

THE PRACTICABILITY OF ROUTINE EXAMINATIONS OF SMALL,
EFFECTIVELY PRESERVED SPECIMENS OF BLOOD DRAWN
BY THE PATIENT¹

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The necessity of maintaining normal blood sugar levels in diabetes mellitus has been urged by a few writers who believe that hyperglycemia, in the treated as in the untreated aglycosuric patient, permits overwork of and resultant damage to the pancreas¹ This goal in treatment conforms with the modern conception of diabetes and with our knowledge of the nature of insulin The necessity of preventing hyperglycemia is not, however, admitted by all writers even though there is practical agreement as to the necessity of keeping patients aglycosuric Some authors consider control of glycemia an unnecessary refinement Many emphasize the importance of blood sugar estimations in diagnosis but consider frequent determinations as a guide in treatment inconvenient and impracticable because of the additional expense and discomfort to the patient These objections have been largely overcome by the introduction of accurate micro methods that permit the use of small samples of capillary blood, easily obtained from the finger or ear, and the use of preservatives, which make unnecessary the immediate examination of specimens That routine determinations with the micro method are practicable has already been demonstrated by Ordway²

The inconvenience to the patient of repeated visits to the laboratory in a fasting state for the purpose of having blood samples drawn seemed to us the chief difficulty in carrying on frequent routine tests In the case of patients who lived at a distance, such examinations were impossible A method was therefore sought which would permit the patient himself to collect small samples of blood that could be effectively preserved and brought or mailed to the laboratory The procedure to be described, adopted after a trial of various micro methods and of preservatives described in the literature, successfully meets these require-

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1 Campbell, W R, and MacLeod, J J R Insulin, *Medicine* 3 262 (Aug) 1924 McCaskey, G W Renal Glucose Threshold and Its Bearing on Insulin Treatment of Diabetes, *New York M J* 118 215-217 (Aug 15) 1923

2 Ordway Tr A Am Phys 39 313, 1924

ments It is an adaptation of methods already reported The Folin-Wu method of blood sugar estimation using 0.1 c.c. of blood, described by Randles and Grigg,³ seemed the most satisfactory of the micro tests and was employed with only slight alteration in technic For preservation the mixture of sodium fluorid and thymol recommended by Sander⁴ was found to be effective It was used in a slightly different proportion in order to insure the presence of sufficient thymol in the smaller amount of mixture required for the micro method

ACCURACY OF THE MICRO METHOD

Randles and Grigg reported that with more than 100 samples run by both the regular Folin-Wu method and the 0.1 c.c. modification the agreement in results was entirely satisfactory, from the clinical point of view at least They found an average difference of about 5 per cent The following slight alterations in technic were made by us The 0.1 c.c. of blood (measured in a 0.1 c.c. pipet made by Emil Greiner for the Kramer-Gittleman micro method) was expelled into the bottom of the centrifuge tube containing the sulphuric acid solution and the pipet was washed out several times with the acid After being stirred, and with the stirring rod still in place, the sodium tungstate solution was added by placing the tip of the pipet on the stirring rod near the surface of the acid

Readings obtained in our laboratory with the micro method as compared with the regular Folin-Wu method appear in Table 1

For values less than 100 mg. per hundred cubic centimeters of blood, the average difference was 0.0066 gm., for values between 100 and 200 mg., the difference averaged 0.0056 gm., and for values between 200 and 400 mg. per hundred cubic centimeters of blood, the average difference in glucose was 0.0077 gm. per hundred cubic centimeters of blood The micro method seems, therefore, to be accurate for clinical purposes as stated by Randles and Grigg

PRESERVATIVE AND ANTICOAGULANT

Randles and Grigg collected their blood samples in vials containing 2 drops of a 1 per cent solution of potassium oxalate which was dried in the vial As a preservative, effective for from two to three days, they used formaldehyd solution added by means of a toothpick dipped in the solution and stirred with the few drops of blood after the excess of formaldehyd had been shaken off

³ Randles, F. S., and Grigg, W. K. Estimations of Blood Sugar by the Folin-Wu Method, Using 0.1 C.c. of Blood, *J. A. M. A.* **82** 684-686 (March 1) 1924

⁴ Sander, F. V. The Preservation of Blood for Chemical Analysis, *J. Biol. Chem.* **58** 1 (Nov.) 1923

More permanent and satisfactory preservation was obtained by us with sodium fluorid and thymol. Sander recommended a mixture of ten parts of sodium fluorid and one part of thymol, the whole having been finely powdered and passed several times through a 100 mesh sieve. Using relatively large amounts of blood, he found that a mixture of 0.01 gm of sodium fluorid and 0.001 gm of thymol to each cubic centimeter of blood was a preservative for from six to fourteen days. For 0.2 c.c. of blood we, at first, used 5 mg of the 10:1 mixture, more than that amount of sodium fluorid was found to interfere with the precipitation of the protein. It was then found with the 10:1 proportion that occasionally a blood was not preserved, probably because

TABLE 1—*Accuracy of the Micro Method*

Regular Folin Wu (2 C c of Blood)	Micro (0.1 C c of Blood)	Difference
0.069	0.062	-0.007
0.075	0.082	+0.007
0.075	0.085	+0.010
0.077	0.069	-0.008
0.079	0.072	-0.007
0.085	0.090	+0.005
0.087	0.074	-0.013
0.088	0.079	-0.009
0.090	0.093	+0.003
0.091	0.083	-0.008
0.097	0.100	+0.003
0.099	0.100	+0.001
0.101	0.102	+0.001
0.102	0.090	-0.012
0.104	0.102	-0.002
0.118	0.118	0.000
0.128	0.136	+0.008
0.128	0.123	-0.005
0.139	0.138	-0.001
0.149	0.154	+0.005
0.161	0.180	+0.019
0.185	0.182	-0.003
0.215	0.220	+0.005
0.222	0.232	+0.010
0.253	0.256	+0.003
0.266	0.270	+0.004
0.323	0.328	+0.005
0.339	0.345	+0.006
0.350	0.334	-0.016
0.357	0.370	+0.013

the mixture, which is a mechanical one, was not complete enough to insure the presence of sufficient thymol in the small amount (5 mg) of the mixture used. The proportion was therefore changed to 10:2, with the result that all of the bloods were perfectly preserved. We recommend this proportion for the use of small amounts of blood.

Table 2 shows the effect of incubation on micro samples taken from the veins of two normal persons. After seven and eight days' incubation, respectively, the final difference was very slight and well within the limits of error of the micro method.

In addition to the preservative series kept in the incubator, micro samples of blood from a diabetic patient were mailed to various parts of

the country after the sugar content was determined Table 3 shows the variations found in the samples on their return to the laboratory from six to eleven days after mailing The differences were well within the limits of error of the method

COLLECTION AND PRESERVATION

Small, wide mouthed vials (7 by 12 mm) fitted tightly with rubber stoppers were used for collecting the samples of blood To these vials had been added from a small glass spoon the mixture of sodium fluorid and thymol Patients were taught to prick their own finger tips with an automatic spring lancet, the point of which had been sharpened and shaped to resemble a Hagedorn needle The points were set at 3 mm

TABLE 2—*Preservation of Bloods Incubated at 37 Degrees Centigrade*

Micro Samples (0.2 Cc) of Same Blood Preserved with 5 Mg of Sodium Fluorid and 0.5 Mg of Thymol			
Before Incubation	Third Day	Seventh Day	Final Difference
0.100	0.091	0.102	+0.002
0.100	0.096	0.098	-0.002
0.104	0.090	0.102	-0.002
0.098	0.098	0.104	+0.006
Micro Samples (0.2 Cc) of Another Blood Preserved with 4 Mg of Sodium Fluorid and About 0.5 Mg of Thymol Added Separately			
Before Incubation	Fifth Day	Eighth Day	Final Difference
0.0865	0.092	0.080	+0.0015
0.091	0.093	0.095	+0.004

TABLE 3—*Preservation of Bloods Sent Through the Mails**

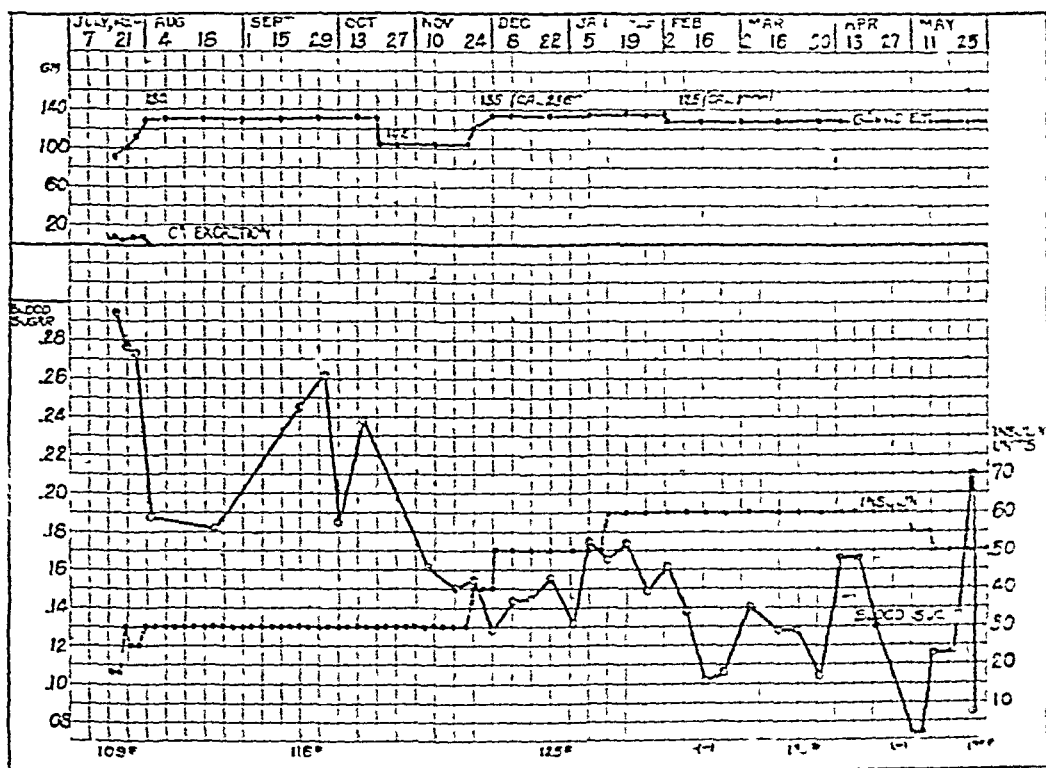
Destination	Days to and from	Blood Sugar (Gm per 100 Cc)
Control (not mailed)	0	0.198
	0	0.206
Hudson, N Y	6	0.192
	6	0.198
Birmingham, Ala	7	0.200
	7	0.204
Los Angeles	9	0.192
	9	0.195
Prineville, Ore	11	0.192
	11	0.197

* Ten micro portions (0.2 cc) of the same blood were preserved with about 5 mg of a 5:1 mixture of sodium fluorid and thymol Duplicate samples were mailed and tested on their return

in order that the resulting cut would insure a free flow of blood By gentle massage of the finger, about 8 drops could usually be obtained After the collection of about 0.2 cc, when the vial was half full, the blood was stirred thoroughly with a tooth pick and the vial stoppered It was then inclosed in a small mailing tube and sent to the laboratory For routine observations bloods were taken by the patients at home before breakfast (and insulin) It was found that even the most nervous patients willingly and easily learned to collect these samples and that the procedure had a valuable disciplinary effect

COMMENT

The practicability of regular, routine estimation of sugar in small samples of blood drawn by patients at home has been demonstrated in a series of diabetic cases during a period of from six to eleven months. The accompanying chart illustrates the clinical course from the standpoint of the fasting blood sugar of one of the patients treated with insulin. Tests were made at weekly intervals for the most part but more frequent readings were made in patients with marked hyperglycemia especially when associated with tuberculosis and other complications. Analyses of the preserved bloods were usually deferred until



Blood sugar curve of patient, aged 31, who was first seen in May 1922 seven weeks after the onset of symptoms. For over a year he remained aglycosuric on a high protein low fat and carbohydrate diet. He then increased his diet to the point of permitting occasional glycosuria. Later after a "cold," glycosuria became constant. In July, 1924, insulin treatment was started and observation of fasting blood sugars was begun. Blood samples were drawn at home by the patient and sent to the laboratory. The thirty units of insulin daily freed the urine of glucose but 60 units were required to maintain a normal blood sugar level. Recently 50 units have sufficed. Temporary elevations have occurred without apparent cause. The weight of the patient has increased from 100 to 137 pounds (45.4 to 62.1 kg).

eight or ten specimens had accumulated. As a result of these determinations a more accurate check on the efficacy of dietary and insulin control has been obtained and variations in metabolism have been more quickly discovered than would have been possible from examination of

the urine alone. The accuracy of the readings is not affected by the utilization of finger instead of venous blood since it has been shown that, in the fasting state, the sugar content of capillary blood, which is virtually arterial, is practically the same as that of venous blood⁵. The procedure described is likewise applicable when more frequent estimations are desired as in carbohydrate and glucose tolerance tests and in all day curves. In the interpretation of such curves, however, consideration must be taken of the fact that the glucose in finger (arterial) blood may be appreciably higher than that of corresponding venous blood⁶.

SUMMARY

Patients can be taught to collect from their fingers and bring or mail to the laboratory a few drops (about 0.2 c.c.) of blood which is effectively preserved and kept from clotting by a mixture of sodium fluorid and thymol recommended by Sander (5 mg. of sodium fluorid and 1 mg. of thymol are used). Estimations of sugar, accurate for clinical purposes, can be made at a convenient time with the micro modification of the Folin-Wu method described by Randles and Grigg. The procedure described is practicable. It provides a method of routine observation of the fasting blood sugar level of diabetic patients.

5 Foster, G. L. Studies on Carbohydrate Metabolism, I, Some Comparisons of Blood Sugar Concentrations in Venous Blood and Finger Blood, *J. Biol. Chem.* **55** 291-301 (Feb.) 1923.

6 Foster (Footnote 5), Cori, C. F., and Cori, G. T. Comparative Study of the Sugar Concentration in Arterial and Venous Blood During Insulin Action, *Am. J. Phys.* **71** 688 (Feb.) 1925.

PANCREATIC FUNCTION

I THE QUANTITATIVE DETERMINATION OF PANCREATIC ENZYMES [†]

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AND

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Enzymes are substances produced by living cells which behave like catalysts in that they change the rate of chemical reactions without themselves undergoing any apparent physical or chemical change. Since enzymes have not been isolated in a pure state, our knowledge of their properties is limited to the study of the reactions that they accelerate or retard.

In quantitative tests of enzymatic activity, a fixed proportion must exist between the amount of enzyme that is present and the effect that is produced. It is therefore essential that the laws governing their velocity of reaction should be properly applied.

According to the law of mass action, the rate of reaction is proportional to the concentration of the reacting molecules. At any moment the velocity of reaction is proportional to the amount of substance that remains undecomposed. In a unimolecular reaction of an inorganic catalyst, such as the hydrolysis of cane sugar by hydrochloric acid,

$$\frac{dx}{dt}, \text{ or velocity of hydrolysis, } = KF(a-x) \quad (1a)$$

a = the initial amount of substrate, x = the amount of substrate that has been hydrolyzed at the moment of observation, k = a constant expressing the ratio between the mass of remaining substrate and the velocity of hydrolysis, and F = the amount of catalyst present. Here the intensity of the reaction is in direct proportion to the concentration of the enzyme.

The relation between the mass of substrate hydrolyzed and the time of hydrolysis is found by integration to be $\log_e \frac{a}{a-x} = K Ft$ (1b). Such a relationship when plotted forms a logarithmic curve.

With enzymes the rate of reaction is not expressed in terms of the equation just given for inorganic catalysts. The velocity of reaction is dependent on many factors, the more important ones being (a) colloidal adsorption, (b) reversibility of reaction, and (c) the effect of the products of the reaction.

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COLLOIDAL ADSORPTION

In the hydrolysis of sucrose by an enzyme, such as invertase, when the proportion of sugar to invertase is high, the rate of hydrolysis is constant for a given amount of invertase. For varying amounts of invertase the velocity of hydrolysis is proportional to the concentration of the enzyme.

Thus, instead of a logarithmic equation, we have

$$\text{Velocity of hydrolysis} = KF \quad (2a)$$

The relation between the quantity of substrate hydrolyzed (x) and the time of hydrolysis (t) must be $x = Kft$ (2b)

Amylopsin in its action on starch follows this law. Here the quantity of substrate changed in any interval of time is a constant amount. The curve of reaction instead of being a logarithmic one becomes a straight line. As the action proceeds, when the substrate is insufficient to bind all of the enzyme, the amount of the substrate hydrolyzed is directly proportional to its concentration, as the logarithmic law requires (Formula 1a and 1b).

This type of reaction can be explained only by the assumption that a compound of enzyme and substrate is formed which exists for an appreciable time (adsorption). All reactions brought about by enzymes occur in a heterogeneous system. The active mass of substrate is unknown. In the hydrolysis of sucrose by acids, the amount of acid that is bound by the sucrose at any instant is so small that it has never been quantitatively determined. In such a case the active mass of substrate is the amount of substrate that is undecomposed.

REVERSIBILITY OF REACTION

All reactions catalyzed by enzymes are reversible. The enzyme combines with the end products of the substrate and then it acts in opposition to its first combination (synthesis). In hydrolytic reactions the rate of hydrolysis is retarded as equilibrium is approached. The velocity of hydrolysis at any moment is the difference between the two opposite reactions of hydrolysis and synthesis, in final equilibrium no apparent change takes place, although in reality the condition is not one of rest but of balance between these two opposing processes each acting at a definite rate.

The rôle of reversibility of reaction is important in the study of the velocity of reactions with steapsin and trypsin. In the case of amylopsin the factor of reversibility is negligible since its hydrolytic reaction progresses almost to completion before any reverse action occurs.

In reversible reactions in which the enzyme is bound and inactivated by the products of hydrolysis, the velocity of reaction will be inversely proportional to the extent of the hydrolysis

$$\frac{dx}{dt}, \text{ or velocity of hydrolysis, } = \frac{KF(a-x)}{x}$$

In the early stages of the reaction, when x is small, $a-x$ is nearly equal to a

$$\frac{dx}{dt}, \text{ or velocity of hydrolysis, } = \frac{KFa}{x} \quad (3a)$$

On integration,

$$X = \sqrt{2KFat} \quad (3b)$$

which is the Schutz-Borissov law, namely, that the intensity of action is directly proportional to the square root of the concentration of the enzyme. This rule holds good, however, only during the early stages of the hydrolysis

EFFECT OF THE PRODUCTS OF HYDROLYSIS

In digestion by trypsin or by steapsin, amino-acids or fatty acids are produced which cause a reduction in hydroxyl ion concentration. This has a destructive action on these enzymes as the optimum hydrogen ion concentration for their activity becomes changed. Hence, it is important in the study of the velocity of reaction of these enzymes to maintain the hydrogen ion concentration at about its optimal value throughout the period of observation.

It follows from the principles discussed that a general law cannot be stated for the relation between the velocity of a reaction and the concentration of an enzyme. In some cases, as in the hydrolysis of sucrose by invertase or in the hydrolysis of starch by amylase, the relation is a direct linear one. Generally, the proportion is expressed in some exponential form as in the square root law of Schutz-Borissov. However, this law is only of limited application during the early stages of certain reactions. The ratio is generally less than the square root.

The only universal rule that has been found to govern the action of the hydrolyzing enzymes is the "time law" of Hedin. The time required to obtain the same effect with varying amounts of an enzyme varies inversely with the concentration of the enzyme. In quantitative determinations of reversible reactions especially, it is more accurate to measure the time taken to effect a definite change than the amount of change in a definite time.

All clinical methods thus far proposed for the quantitative determination of pancreatic ferments have been based on the determination of the amount of enzymatic activity on various substrates at the end of a fixed interval of time (from one to twenty-four hours). As discussed above, only in the case of amylopsin is there a direct linear

proportionality between the amount of enzyme present and the amount of hydrolysis effected, when the substrate is in excess (The velocity of reaction follows equation 2a and 2b) In Table 1 is given the number of cubic centimeters of digestion mixture required to reduce 5 c c of Benedict's copper solution To flasks containing 50 c c of 3 per cent aqueous solution of soluble starch, 1 c c, 0.5 c c, 0.25 c c and 0.125 c c of the same specimen of duodenal contents were added These were incubated for one-half hour at 37.5 degrees C¹

These results, within the limits of experimental error, follow the law of direct linear proportionality Benedict's solution is reduced

TABLE 1—Cubic Centimeters of Starch Digestion Mixture Required to Reduce Five Cubic Centimeters of Benedict's Solution

Amount of Duodenal Contents Used	Case 1	Case 2	Case 3	Case 4	Case 5
1 c c	0.82	0.85	1.30	1.20	1.30
0.5 c c	1.48	1.70	2.60	2.30	2.65
0.25 c c	2.56	3.00	5.30	4.40	5.65
0.125 c c	5.28	6.05	11.45	9.00	11.60

TABLE 2—Determination of Tryptic Activity (Method of Lueders, Bergem and Rehfuß)

Amount of Duodenal Contents Used	Amount of Tenth Normal Sodium Hydroxid Required to Bring Digestion Mixture to Neutral Point				
	Case 1	Case 2	Case 3	Case 4	Case 5
1 c c	6.1	6.5	6.0	7.2	3.7
0.5 c c	5.8	5.6	4.8	5.7	2.6
0.25 c c	3.9	3.3	3.7	4.3	1.3

TABLE 3—Determination of Lipolytic Activity (Method of Lueders, Bergem and Rehfuß)

Amount of Duodenal Contents Used	Amount of Tenth Normal Sodium Hydroxid Required to Bring Digestion Mixture to Neutral Point				
	Case 1	Case 2	Case 3	Case 4	Case 5
1 c c	9.0	9.3	1.9	5.0	5.5
0.5 c c	6.0	5.2	1.0	4.0	4.1
0.25 c c	4.3	4.1	0.7	3.2	2.6

only by the maltose formed The fact that the hydrolysis of starch occurs in stages, with the formation of several dextrans as well as maltose, does not produce any appreciable error

In the case of trypsin (Table 2) and of steapsin (Table 3) no simple proportionality was found between the amount of enzyme used and the amount of hydrolysis effected This is in accord with the

¹ To stop further activity of the amylase, a small amount of sodium carbonate should be added to each flask In the reduction of Benedict's solution, the digestion mixture can be added rapidly till a milky precipitate appears Further addition should be by drops with constant boiling for several seconds between each drop

theoretical analysis of the behavior of these enzymes discussed above. The method used was that of Lueders, Bergeim and Rehfuess, which was considered the best clinical method recently proposed. The method recently advanced by McClure, in which buffers are used to maintain a constant hydrogen ion concentration throughout the reaction, was considered too complicated and time consuming to be of clinical value and was therefore not employed. In the method of Lueders, Bergeim and Rehfuess, lipase and steapsin act on a 20 per cent emulsion of olive oil and on a 5 per cent aqueous solution of gelatin, respectively, for one hour. The amount of fatty acids or amino-acids formed is determined by titration with tenth normal sodium hydroxid.

To obtain a simple and exact relationship in the quantitative determination of trypsin and steapsin we have applied the "time law" of Hedin in the following manner. Trypsin and steapsin are both active in a neutral or a slightly alkaline medium. Phenolphthalein begins to turn red at a p_H of 8.4. If 1 c.c. of tenth normal sodium hydroxid is then added to 25 c.c. of the substrate, a deep red results and the hydrogen ion concentration is not changed sufficiently to inhibit the enzyme. The time required for the hydrolytic activity (amino-acid or fatty acid formation) to neutralize this 1 c.c. of tenth normal sodium hydroxid and cause the disappearance of all pink in the substrate is in inverse ratio to the concentration of the enzyme.

With steapsin (Table 4) 10 per cent olive oil emulsion² was used as substrate. To 200 c.c. of olive oil emulsion, 2 c.c. of 1 per cent alcoholic solution of phenolphthalein is added by the addition of tenth normal sodium hydroxid the emulsion is brought to a permanent light pink. Twenty-five cubic centimeters of this neutralized oil is placed in each of eight 30 c.c. test tubes of uniform size. To each of four of these tubes 1 c.c. of tenth normal sodium hydroxid is added, which produces a deep red. The four remaining test tubes containing 25 c.c. of neutralized oil are used as controls. The tubes are placed in a water bath at 37.5 degrees C., and this temperature is maintained throughout the period of observation. To each tube and its control the same amount of duodenal contents is added from a 1 c.c. pipet graduated to 0.01 c.c. The pipet is inserted to the bottom of the tube and its contents are gently blown into the substrate of oil while the pipet is being withdrawn. In this way practically none of the ferment is lost. The test tube is then shaken a few times by hand. The time required for the disappearance of all pink by comparison with the control tube

² To make olive oil emulsion (10 per cent) 50 gm. of gum arabic should be placed in a mortar and 50 c.c. of pure olive oil added and rubbed into a smooth paste. Then 50 c.c. of distilled water should be added and constantly stirred until white emulsion is formed. More water, to a total volume of 500 c.c. should be gradually added. This should be kept in an icebox.

(neutralization of 1 c c of tenth normal sodium hydroxid by the fatty acids developed) is determined in seconds and minutes. When the pink is gone the tube is of a slightly lighter yellow than the control tube, owing to the extra 1 c c of tenth normal sodium hydroxid that was

TABLE 4—*Time Required for Disappearance of All Pink (Neutralization of 1 C c of Tenth Normal Sodium Hydroxid by Fatty Acids)*

Amount of Duodenal Contents Used	Case 1	Case 2	Case 3	Case 4	Case 5
0.4 c c	$\frac{1}{2}$ min	2 min	$\frac{3}{4}$ min	$\frac{1}{2}$ min	50 sec.
0.2 c c	$1\frac{1}{4}$ min	4 min	$1\frac{1}{2}$ min	$1\frac{1}{4}$ min	2 min
0.1 c c	$2\frac{1}{2}$ min	9 min	3 min	$2\frac{1}{4}$ min	5 min
0.05 c c	6 min	17 min	6 min	$5\frac{1}{2}$ min	$10\frac{1}{2}$ min

TABLE 5—*Time Required for Disappearance of All Pink (Neutralization of 1 C c of Tenth Normal Sodium Hydroxid by Amino-Acids)*

Amount of Duodenal Contents Used	Case 1	Case 2	Case 3	Case 4	Case 5
1 c c	3 min	$1\frac{1}{2}$ min	10 min	7 min	3 min
0.5 c c	6 min	3 min	20 min	13 min	6 min
0.25 c c	12 min	7 min	41 min	30 min	13 min
0.125 c c	24 min	14 min	Not determined	57 min	25 min

Protocol of Case 1

Amount of Duodenal Contents Used	Minutes After Addition of Enzyme to Substrate	Remarks
1 c c added when second hand of watch was at 60	1	Red
	1	Pink
	1	All pink gone
	1	All pink gone (no further change)
Conclusion Reaction complete in three minutes		
0.5 c c added when second hand of watch was at 60	3	Red
	1	Pink
	1	Light pink
	1	All pink gone
Conclusion Reaction complete in six minutes	1	All pink gone (no further change)
0.25 c c added when second hand of watch was at 60	6	Red
	2	Pink
	3	Light pink
	1	All pink gone
	1	All pink gone (no further change)
	1	All pink gone (no further change)
Conclusion Reaction complete in twelve minutes		
0.125 c c added when second hand of watch was at 60	7	Red
	8	Pink
	4	Light pink
	2	Faint pink
	1	Faint pink by comparison with control
	1	Faint pink by comparison with control
	1	All pink gone
	1	No further change
Conclusion Reaction complete in 24 minutes		

added. In the tubes with the smaller quantities of enzymes the pink changes slowly. In these, the reaction is watched for a few minutes after all the pink has disappeared to make certain that no further change occurs (Case 1, Table 5).

With trypsin (Table 5) 5 per cent gelatin in an emulsion of mineral oil³ was used as substrate. The same technic was employed as described for steapsin, except that larger amounts of duodenal contents were used.

Within the limits of experimental error, these tables show that the time required to neutralize 1 c.c. of tenth normal sodium hydroxide is in inverse ratio to the amount of ferment present. The clinical application of these methods will be the subject of a future report.

SUMMARY

1 Present clinical methods for the quantitative determination of pancreatic ferments are based on the determination of the amount of enzymatic activity obtained at the end of a fixed interval of time (from one to twenty-four hours).

2 However, in reversible hydrolytic reactions no definite proportionality exists between the velocity of a reaction and the concentration of an enzyme. Only in the case of amylase, in which the factor of reversibility is negligible, does a direct linear proportionality exist between the amount of enzyme present and the amount of hydrolysis effected.

3 In reactions with trypsin and steapsin a linear proportionality can be obtained by the application of the "time law" of Hedim. The time required to obtain the same effect with varying amounts of an enzyme varies inversely with the concentration of the enzyme.

4 We have applied the "time law" of Hedim in a simple manner that can be employed in quantitative tests for these ferments.

3 Gelatine emulsion (5 per cent) is milky white and forms an excellent contrast medium for the pink of the phenolphthalein. Aqueous gelatin solution is light brown and does not contrast well with a pink. To make the emulsion 25 gm. of highest grade gelatin should be added to 300 c.c. of distilled water and gradually heated to 60 degrees C. till the gelatin is completely dissolved. Fifty grams of best grade (whitest grade) gum arabic and 50 c.c. of liquid petrolatum are placed in a mortar and rubbed into a smooth paste, then 50 c.c. of distilled water is gradually added with continual stirring. When a homogeneous paste is formed the heated gelatin solution should be added gradually and stirred continually. A milky white emulsion is then formed. Hot distilled water should be added slowly till the total volume is 500 c.c. This should be kept in the icebox in 50 c.c. flasks. When needed these are liquefied by gentle heat in a water bath.

Book Review

CLINICAL FEATURES OF HEART DISEASE AN INTERPRFTATION OF THE MECHANICS OF DIAGNOSIS FOR PRACTITIONERS By LEROY CRUMMER, M D, Professor of Medicine, University of Nebraska With an introduction by EMANUEL LIBMAN, M D, Physician to Mount Sinai Hospital, Professor of Clinical Medicine, Columbia University, New York New York Paul B Hoeber, 1925

Few books have been written in recent years on disease of the heart in which considerable space has not been devoted to the newer methods in cardiac diagnosis Not infrequently there has been a tendency to overestimate the value of the instruments of precision This book by Crummer, with introduction by Libman, is an exception to the general tendency in that the mechanical aids employed in cardiac diagnosis are considered in a short chapter of fifteen pages

Emphasis is placed on the value of the simple methods of physical examination and careful clinical observations and at this time this fully justifies a work of this type The book is apparently not intended to make an extensive survey of the entire field A creditable balance is, however, maintained and several suggestions are made which should stimulate further careful clinical observation The book is easily read It will be a helpful guide to the practitioner and of interest and value to those particularly interested in cardiac disease

PERIPHERAL PULSATIONS IN THE VEINS IN CONGESTIVE FAILURE OF THE HEART, ASSOCIATED WITH PULSATION OF THE LIVER AND TRICUSPID REGURGITATION¹

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Our purpose is to offer a means of arriving at an early and more definite knowledge of cardiac incompetency by calling attention to certain well marked pulsations in the peripheral veins. These are especially pronounced in the median basilic vein of the forearm and in the veins on the dorsum of the hands, and they occurred in all of a series of more than fifty cases in which a diagnosis of cardiac failure and tricuspid insufficiency had been made on other clinical and anatomic grounds. A large number of compensated controls did not show these pulsations.

The relatively common occurrence of functional tricuspid insufficiency is not recognized by clinicians in general as often as it should be. This has been pointed out frequently by Mackenzie.¹ This fact was brought home to us when it was discovered that quite a large proportion of the average run of patients at our disposal in the medical and surgical wards had some degree of functional insufficiency though in the great majority of cases this was not suspected at the time. When this fact was determined and the patient was given supportive treatment, there was a decided improvement in prognosis.

Venous pulsations in the jugular veins is the subject of a large amount of literature. This literature deals in part with tricuspid insufficiency, its signs and symptoms in diagnosis, and in part with the interpretation of the graphic registration of venous pulsation. There are only a few isolated references to pulsations in the peripheral veins. These references have not been given proper prominence nor has their significance been recognized in relation to cardiac insufficiency. No single author, so far as we were able to ascertain, has ever definitely compiled a series of cases of venous pulsations in the peripheral veins with the idea of indicating their almost constant association with tricuspid insufficiency or their early appearance with the onset of congestive failure. Aside from their historical interest, these reports are

¹From the Department of Medicine, University of California Medical School.

¹ Mackenzie. *Diseases of the Heart* 1914, p. 336.

valuable in that they are accurate descriptions of cases comparable to some that we have observed. We quote at some length from certain early articles

HISTORICAL REVIEW

Davis² in 1827 (also cited by King and others) reported a case of "remarkable pulsations in the veins" as follows

"June 21, 1826, Margaret Connor, act 6, emaciated, whooping cough four months previously with poor recovery, vomiting for last ten days. Pulse 88, slightly irregular, full and pretty strong, constipated, petechiae resembling purpura simplex and a milium vesicular eruption on chest and neck"

"Pulsations in all the veins, distinct and well marked, synchronous with that of the arteries and in the veins of the extremities, perceptible to the eye even at a distance of 2 yards. The veins were rather larger than is usual at her period of life and pressure upon any of them stopped the pulsation between the point compressed and the heart, so that it obviously could not be caused by regurgitation from the auricle." Necropsy showed hydro-pericardium and "the left ventricle of the heart was somewhat enlarged and firmer than natural." The rest was normal. The arteries were injected and showed no enlarged communications with the veins and no arteries were found near these veins "which could throw a doubt on the fact that the pulsation had been continued through the arteries and capillaries to the veins"

Ward³ in 1832 reported a "singular effect of castor oil"

A woman, aged 30, five months pregnant, had fever, cough and generalized pains, dyspnea, rapid pulse and exhaustion from catharsis and bleeding. Three days following miscarriage, Ward says, "she was sitting up, and I observed that the veins of her hands were greatly distended and pulsated with great violence. The skin being very pellucid, the pulse was very distinct in the small ramifications of the fingers, in which the blood was of the arterial color, the larger veins being darker but not so blue as usual. The pulsation extended beyond the middle of the forearm and was not stopped by pressure upon the veins at any point above, though it was stopped by pressure below the pulsating parts whether on fingers or back of hand." It was synchronous with the radial pulse which beat high. The pulsations continued for three days and gradually disappeared, after which there was some transitory edema of the legs. He thought that the "excessive reaction of the heart pushing the thin and impoverished blood through the capillaries right on into the veins" overcame the restrictions of the capillary bed.

Graves⁴ (1833), cited by King, saw a patient with Dr Ireland and Mr Crampton. He says

She had a violent attack of peritonitis and during the course of her illness we observed this leaping of the arteries, accompanied by a visible pulsation in the veins on the back of the hand, yet both entirely ceased when the inflammation subsided.

Benson⁵ (1836), in a comprehensive paper reported a case of venous pulsation in the upper extremities, on section he determined to

2 Davis Dublin Hosp Rep 4 272-275, 1827

3 Ward London M Gaz 10 376, 1832

4 Graves London M & S J 3 77, 1833

5 Benson Dublin J Med & Chem Sc 8 324-332 1836

his own satisfaction "that hypertrophy with dilatation of the right ventricle was the true cause" The following is a brief resumé of the case

Mary Oliver, aged 60, was alcoholic, weak, pale and emaciated There were marked pulsation of the heart and loud murmurs over all the precordia She became comatose and pulsations were noted in "every superficial vein of the two upper extremities" The pulsations in the distended veins were synchronous with the radial pulsations though a little later The veins of the trunk and lower extremities were motionless The jugular and carotid veins beat vehemently He says that the 'internal jugulars became greatly distended and collapsed during each act of respiration, whilst a confused tremulous pulse incessantly agitated them" Venous section of 10 ounces (295 cc) relieved the patient and caused the "pulsations to disappear The blood did not come per saltum although pulsation was observed in some of the veins below the bandage" Four days later the pulsations returned

"My colleagues and the hospital pupils now joined me in observing the pulsations and the veins were seen to rise and fall with respiration, becoming turgid towards the end of respiration and flaccid when inspiration was nearly complete In both states, however, their pulsation went on regularly, beating as often as the artery, eighty in a minute, but a little after it There was no doubt of this, though the interval was very minute" On venous section from the cephalic vein to obviate arterial pulsations, the blood "flowed distinctly per saltum It was like arterial blood in color but of much thinner consistency" "After removal of eight ounces, the pulsations ceased" The patient died the next night

Before section, there were two opinions as to the cause of the pulsating veins These may be presented in full because they point out the situation as it stands today, and the proof that Benson offers for his contention holds equally well now Furthermore, it is similar to that offered by us (some eighty-seven years later)

"First, that the pulsation was derived from the left side of the heart, sending on its blood through the capillaries into the veins, second, that it was derived from the right side of the heart, and depended on regurgitation" "In support of the former opinion, it was observed, first, that pressure on the brachial or subclavian arteries stopped the pulsation, but that it was unaffected by pressing on a vein, second, that the valves acted perfectly as could be seen by emptying a portion of vein just below a valve, and therefore that regurgitation was impossible, third, that the venous pulse occurred at such an interval after the arterial as was inconsistent with regurgitation, but might be expected if the blood had to travel round by the capillaries, and fourth that the debilitated condition of the patient might have so relaxed the capillaries as to allow of an unusually free communication between the arteries and veins"

"In answer to these arguments and in favor of regurgitation, it was urged, first, that pressure on the brachial or subclavian arteries also acted more or less on the principal veins which accompanied them, besides by diminishing the quantity of fluid which the veins received it would render their pulsation less evident, in the same way that the bleeding did, but that the free anastomosis would make amends for the slight interruption occasioned by pressing on a superficial vein second, that though the valves acted well when the vein was emptied behind them, they would not prevent an impulse from being conveyed along the column of blood in a distended vessel and that, in fact, if each valve were merely thrown suddenly across the venous tube it would cause a venous pulse, though ever so perfect in its valvular function, third, that the yielding coats of the veins would serve to retard the rapidity of the returning wave and thus account for the momentary delay in their beat, and fourth that the arterial pulse was not strong enough to justify us in supposing that it could be continued on to the veins, nor was there any ground for saying that the capillaries were relaxed"

At section, twelve hours after death, there were found generalized fibrous adhesions of the pleura, the lungs were free from disease and not even congested. The heart was markedly dilated and hypertrophied to twice the usual size. "The right auricle was dilated and a little hypertrophied. The right auriculoventricular opening was very large and gaping. The right ventricle was dilated and hypertrophied. Its cavity was twice as large and its walls twice as thick as usual. The floating margins of the tricuspid valves were thickened and studded with small cartilaginous nodules. The pulmonary valves were slightly thickened and the left auricle and ventricle slightly dilated and thickened. There was marked mitral stenosis with calcareous deposits. Thickened insufficient aortic valves. General atheroma. Abdominal viscera were healthy." The venous valves were competent, the weak left ventricle, in his opinion, could not have forced blood past the obstructions of the aortic valves and the calcareous aorta through the capillaries to cause the veins to pulsate. The pulse was not "from the right auricle, for their diastole always followed, though at an exceedingly short interval, the diastole of the arteries." It was not from the "coats of the veins" nor in the neighboring arteries." He suggests that an hypertrophied right ventricle and a dilated auriculoventricular opening would allow regurgitation into the auricle of considerable force to communicate an impulse to the dilated veins "even to their small ramifications." The propagation of their impulse depended on the distended veins and not on the insufficiency of their valves. Benson points out that the interpretation of this and other cases reported (most of which are reviewed here) was fundamentally wrong and that "the dissection proved, I think, incontestably that hypertrophy with dilatation of the right ventricle was the true cause" (p 331).

King⁶ (1837), in his "Essay on the Safety Valve Function in the Right Ventricle of the Human Heart," makes several personal observations on pulsating veins. As these observations are unique, we are justified in quoting at some length. In his paragraph, "Venous Pulse in Health and Disease" (p 107), he points out that on immersing the hand in warm water a venous pulsation equal in regularity and number if not synchronous with the arterial pulse can be seen in the cephalic vein. This pulsation causes an increase of the jet during venous section with each arterial pulsation. He suspects the capillary circulation of allowing the ventricular systole to penetrate to the veins (p 106), i e., "that the chief force is the ventricular impulse, through the arterial tubes and capillaries, into the veins of every part."

He says, "I was, some time since, surprised to find, that upon lying on my back in the sunlight, after dinner, the large mesian veins of my forehead were visibly pulsating. They are naturally of full size, and having become distended, a rather slow and slight diastole was seen, by the aid of a mirror, with or a little later than each temporal pulse." He naively explains in a footnote that he had had a moderate dinner "with one glass of porter and nearly as much water" one-half hour before. He repeated the observation and said, "Having taken water only, my veins still beat." Another time, while hungry, he found it necessary to elevate his feet to bring out the pulsation in the engorged veins. (This is rather an expose of his appetite.)

⁶ King. Guy's Hosp Rep 2 104-178, 1837

To obviate the charge of the "will to see," he has had others beside himself verify these pulsations in several cases. "On more than one occasion of unusual vascular excitement, I have also seen the dorsal veins of my own hands beating"—also of the leg and foot (p 109). He affixed a waxed thread 2 inches (5 cm) long with a little tallow to the veins of the hand and demonstrated the pulsations as differing markedly from the arterial beating. "In some veins, which, by a semirotation of the hand may be placed either in front or posteriorly, the effect has been the same," i e, he has tried to rule out the effect of an adjacent artery. By means of this capillary level, he determined that the slow pulsations in a small superficial vein were manifestly later than the quick radial pulse.

In the pursuit of the same study, I was attending Dr. Bright in his visits to the wards of the hospital, and found two men with visibly pulsating veins on the backs of their hands. The doctor not only saw, but thought he could feel, the venous diastole, the wrist being all the while embraced with some firmness to render the veins large. The patient had an hypertrophic heart and a very powerful pulse, his veins were rather few. In the second instance,

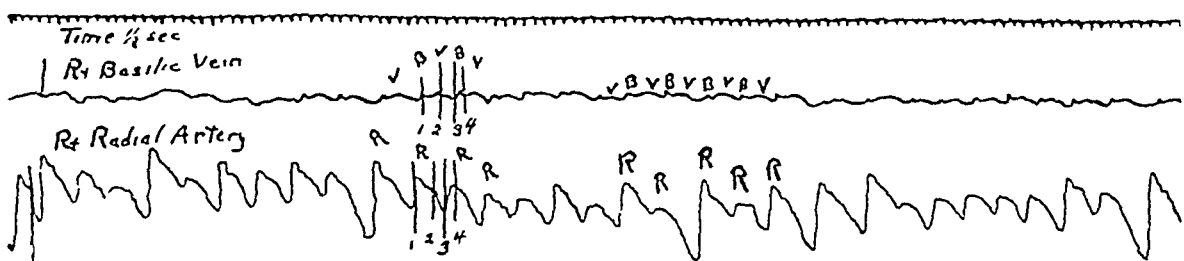


Fig 1 (Case 1)—Tracings obtained with glass capsule on median basilic vein in mitral stenosis with fibrillation. 1, brachial and radial systole practically synchronous, B, wave transmitted from brachial artery similar to C wave in jugular pulse, 2, peak of venous pulsation in diastole of radial pulse, V, similar to V wave of jugular pulse, 3, end of venous pulsation and beginning of systole, and 4, beginning of venous pulsation at time of middle of radial systole (approximately one-fifth second after beginning of systole). In some tracings the brachial artery wave is absent. This is best shown when the skin is kept loose and the capsule applied as far from the pulsating artery as possible.

which was less marked, the man had had renal anasarca for sixteen days. His heart probably was not quite sound. The pulse was soft and tolerably good. He was perspiring profusely. The dorsal veins of the hands were pretty numerous and large and they were covered with abundance of hair, which was remarkable for the reason that the veins not only beat visibly in many places, but that particular hairs, which were well adapted for the purpose, seemed to form so many natural sphygmometers. It was here also necessary to enlarge the veins by gently grasping the wrist.

He cites the case of a girl, aged 11 years, with purpura and fever, during which, he says, "on very gently grasping the wrist in feeling the pulse, I observed for several days the numerous and rather full veins of the hand both prominent and beating. There was in all of them a very distinct diastole and systole." The patient died shortly after. The

lungs were edematous the "heart's substance healthy," the blood watery. There were pale hypertrophic kidneys and suppuration of the parotid.

King was the first to compile the cases in which venous pulsations of the extremities had been previously noted. He says, "The earliest notice I have been able to find of a similar case is that which Haller (*Elementa Physiologiae*, T II, p 256) has quoted, and single instances have been more recently related by Steinbuch (*Id pract Heilkunde*, September 1815), in the *Philadelphia Journal* (Graves), by Sundelin (*Arch f Med Erfahr* 1822), by Beyer (*J Complimentaire*, T XXI, 1825), by Davis² of Dublin by Elliston (*Blumenbach's Physiology*, last edition), by Ward,³ by Graves⁴ (who indeed records two), and lastly by Benson"⁵

Bernard⁷ (1862), during stimulation of the lingual nerve, noted that the blood vessels of the submaxillary gland dilated and the pulse could be seen in the capillaries and veins and that the blood flowed from the veins bright and red and spurting, as if from an artery. This was probably a true relaxation of the capillary bed. Such a condition might occur in the superficial veins of the hands, etc., but usually with the venous congestion of tricuspid insufficiency there is a low blood pressure because of the weakened myocardium. They could arise from some vasomotor disturbance which should be found independently of tricuspid insufficiency.

Friedreich⁸ (1866) noted simple pulsation of the left forehead and temple veins, vena cephalica antibrachii dextra, and the retinal veins in a case in which necropsy showed, besides other pathologic conditions, a dilated and large right heart with sclerotic insufficient tricuspid valves. He suggested that tricuspid insufficiency in this case was first accompanied by a full and pulsating liver and later by the engorgement and reflux overcoming the venous valves, and that the pulsations appeared in the jugular bulb, the external jugular and finally in the vena cephalica antibrachii dextra. The vein valves were in good shape at necropsy. He considered liver pulsations as pathognomonic for tricuspid insufficiency. He saw pulsating veins on the forehead in a similar case in which on section a remarkable dilatation of the vena cava as well as a dilated right auricle were present.

He reviewed the literature thoroughly for venous pulsations in the extremities (p 275) and cites observations of such pulsations by Homberg, Zuhm and Elliotson. He remarked that pulsations in the facial veins had not been mentioned before his two cases were reported. This is the first mention of pulsations in the retinal veins in this connection. He also cites Mavey, who, with Goubler and Verneul, noted pulsation in varicose veins of one leg. In 1867, Friedreich⁹ cited Walshe as having

7 Bernard J de Physiol et Path 5 383-418 1862, 1 233, 649 658, 1858

8 Friedreich Handb d spec Path u Therap 5 192, 1867

9 Friedreich Deutsch Arch f klin Med 1 241-291, 1866

noted pulsations in the vena mammae. He thought the reason for the more common appearance of marked jugular pulsations on the right side was the position of the right innominate trunk.

Quincke¹⁰ in 1868 described pulsating veins in the hands and feet of an anemic girl, the forearm and hands of a normal youth with a cord injury which had caused vasodilatation of the arms, a case of cholelithiasis in a woman, aged 50, with aortic insufficiency and hypertension, and on his own hands when the veins were dilated by heat. His criteria for seeing these pulsations are a thin transparent skin free from edema and well filled veins that stand out well. He mentioned pulsations in the vena centralis retinae as commonly seen. In the case of vasodilatation the pulsations may well be transmitted from the arterioles as in Bernard's observation.

Friedreich¹¹ (1878) says, "Ich glaube den cruralvenenklappen-ton für ein ebenso sicheres Zeichen für die Diagnose einer Insufficienz der Tricuspidal Klappe, betrachten zu können, als den früher von mir

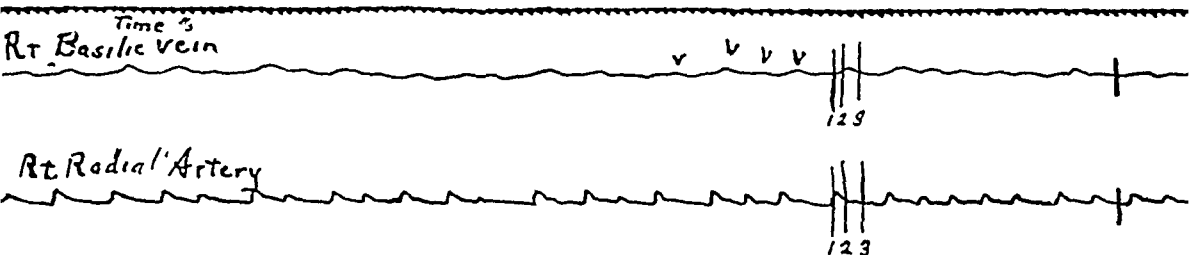


Fig 2 (Case 1) —Tracing obtained with intravenous needle in median basilic vein 1, beginning of radial systole, absence of brachial artery pulsation in absolutely smooth venous curves, tambour levers show only one pulsation, auricular waves are absent as fibrillation is present 2 diastolic notch just after the beginning of the venous wave, 3, end of radial pulse wave just before the end of the venous wave, with the summit between 2 and 3

beschriebenen Lebervenenpuls." He describes several cases characteristic of mitral and tricuspid insufficiency, all of which showed the venous double tone in the "crural" vein and one of which presented a markedly pulsating median vein of the forearm. The first "crural" tone he thinks is due to the auricular systole and the second to ventricular reflux—they are accentuated and brought out by light pressure with the stethoscope.

Gerhardt¹² in 1894 noted a few pulsating veins over the manubrium in a case of mitral and tricuspid insufficiency with pulsating liver, pulsations in the crural veins and edema. In 1902¹³ he was of the opinion that diastolic collapse is occasionally seen in the small

10 Quincke Berl klin Wchnschr **34** 357, 1868

11 Friedreich Arch f klin Med **21** 205-258, 1878

12 Gerhardt Arch f exper Path u Pharmakol **34** 402-405, 1894

13 Gerhardt Arch f exper Path **47** 250-266, 1902

veins, i. e. external jugular, branches of the external mamillary and, seldom, the arm veins, while the great veins show systolic pulsation

Gaertner¹⁴ in 1903 frequently observed venous pulsations while reading venous pressures by his method

Babcock¹⁵ (1903), in his chapter on aortic regurgitation, spoke about a slow undulating venous pulse in the superior veins of the hand and forearm when they hang down so that the veins are turgid. This is especially prominent when arterial tension is low, as in fevers, anemia, etc

Sewall¹⁶ in 1906 stated that the veins of the forearm swell and contract with respiration and the pulse beat, and that a paper lever applied across them will show the oscillations

Preble¹⁷ in 1906 noted pulsations in the veins on the chest wall and upper arm in three patients with pernicious anemia who died soon afterward. The liver was not much enlarged. There was moderate dilatation of the heart and slight edema of the legs. No murmur was heard in any of the three cases

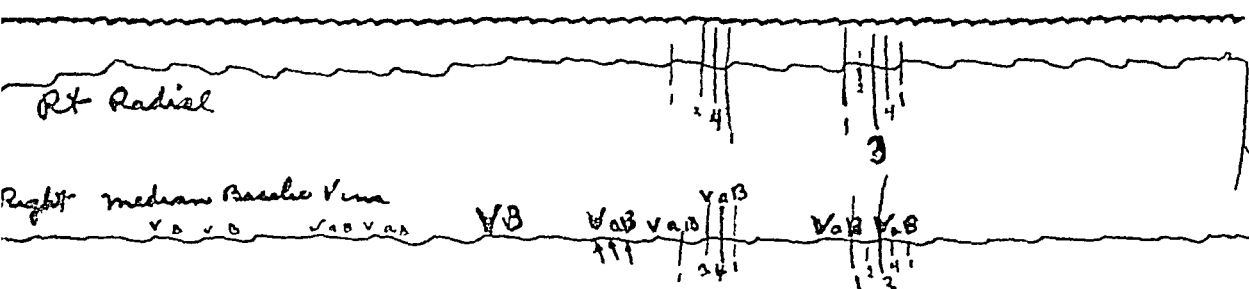


Fig 3 (Case 2) —The pulsations obtained with the glass capsule on the basilar veins show three waves, comparable with the type of jugular pulse (Figs 4 and 5). 1, the short systolic wave which must be that due to the brachial artery and similar to the *C* wave in the jugular pulse is hidden in the end of the long wave and occurs at the same time as the radial systole, 2, the diastolic notch of the radial systole is at the same time as the beginning of the venous, or *V*, wave (Fig 2), and 3, is on the crest of the wave after the diastolic notch of the radial pulse and on the crest of the venous wave, *V*, or the same as 2 in Figure 2 and 3 in Figure 6, 4, the little waves seen to occur between *V B* and occasionally registered on the tracing must be the *A* waves as they are at the interval in which they appear on the jugular tracings $AV \approx$ about $2/5$ second $VA \approx$ less than $1/5$ second

The pulsations seen by Staehelm and Ortner¹⁸ (1913) in cases of tricuspid insufficiency were in the jugular vein and bulb, the liver (in which the wave may be earlier than in the neck), and the skin veins of the lower extremities. They noted that pulsations are especially marked in the neck if the liver is compressed

14 Gaertner *Munchen med Wchnschr* 50 2038-2041, 1903

15 Babcock *Diseases of the Heart and Arterial System*, 1903

16 Sewall, H. *J A M A* 47 1279 (Oct 20) 1906

17 Preble *Am J Med Sc* 132 393, 1906

18 Staehelm and Ortner, in Krause *Klinische Diagnose*, 1913, pp 219 and 252-253

Pezzi and Gasperini¹⁹ (1914) reported three cases with A and V waves in the liver and femoral vein tracings as characteristic of cases of tricuspid insufficiency

Wilson,²⁰ in Nelson's System (1920), said that "the peripheral veins sometimes pulsate in exophthalmic goiter but venous pulsation is ordinarily confined to the internal and external jugular veins"

Hill²¹ in 1921, in studying the pressures in the small arteries capillaries and veins of a bat's wing, said, "A slow, rhythmic pulsation, as is well known helps to maintain the flow in the veins of the bat's wing This is little evident in the hot blooded state when the velocity of flow in the arteries and veins is very fast"

It would appear, therefore, that in offering our interpretation of pulsations in peripheral veins in a large series of cases, we are following the lead of numerous observations in the literature It is striking that these observations should have been reported most accurately at a time when the practice of medicine was more of an art and less dependent on routine laboratory technic At that time also venesection brought more attention to the veins than is usual at the present time

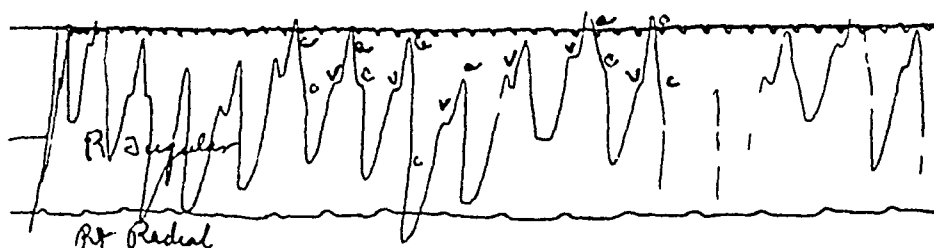


Fig 4 (Case 2) —Ventricular type of jugular venous pulse, systolic blood pressure 100, diastolic 84, radial rate 100-90, taken just after Figure 3 AV \approx about 2/5 second VA \approx less than 1/5 second

METHOD

The patient is usually in a semi-Fowler position or flat on his back in bed The light should be adjusted so as to come from the side Daylight is preferable to artificial light unless a fairly strong diffuse white light can be obtained The observer should be seated comfortably on a chair drawn up to the bedside The patient's arm should be held in moderate abduction and supported at the elbow and wrist by allowing it to rest on the palms of the hands Raising the elbow gradually while flexing the forearm gently back and forth on the arm will soon enable one to find the level of collapse of the veins at any point The "critical point" of venous collapse is a rough estimate of the venous pressure (method of Gaertner) and thus an index to the relative amount of venous congestion

19 Pezzi and Gasperini Arch mal du cœur 7 593-608, 1914

20 Wilson Nelson's Loose-Leaf Living Medicine 4 415, 1920

21 Hill, A V Proc Phys Soc, February, 1921, J Physiol 54 332 (March) 1921

The large branches of the basilic vein seem best fitted for making these observations as they are in an area where the skin is thin somewhat transparent and usually devoid of much fat or edema. The basilic vein is usually made up of three branches which unite in the region of the median condyle on the ventral or mesial surface of the cubital fossa.

In positive cases the pulsations vary from a mere flicker to large excursions of from 2 to 3 cm., depending on the state of engorgement and the degree of insufficiency. In doubtful cases, if the forearm is kept only slightly flexed so that the surface of the venous level is spread along a centimeter or so of vein, the faint flicker may be more easily detected. Also, if the veins are stripped proximally, by light massage with the finger the pulsations may be more readily identified immediately after each stroke.

Obviously, this method is not applicable to the obese with veins buried in fat, in women with very small veins, in cases with regional edema or in which the veins have been obliterated by thrombi or venous section or obscured by subcutaneous hemorrhage or injections. The pulsations may be magnified by placing a strip of paper or a thread or by observing conveniently placed hairs (the sphygmometer of King).

Tracings of these pulsations are obtained by means of a glass capsule connected with a sensitive polygraph. The capsule should be slightly elongated and shaped to fit the arm over the vein. It is fastened in place by collodion and should not obstruct the course of the pulsations beneath it. The best excursions of the writing pen are obtained by causing the collapsing point of the venous column to fall just beneath the capsule. The registration of these pulsations requires some delicacy of adjustment and much practice, as they have nowhere near the volume nor the force of the jugular pulse. Synchronous radial, jugular or apex tracings are also taken.

In normal individuals and controls, we have been unsuccessful in seeing or obtaining pulsations from the vein in this manner, but occasionally a small sharp systolic wave is obtained from the brachial artery nearby. The collapsing point of the veins was noted, usually with the patient flat on his back if possible, or in relation to the xiphisternum or fifth costochondrial junction (Gaertner) in semi-Fowler position.

Occasionally, the venous pressure was measured by a method slightly modified from that of Hooker²² or directly with a water manometer by means of a large intravenous needle in the vein. Several tracings of the pulsations were obtained from the manometer during the latter procedure. The usual notations on the size of the heart, murmurs, blood pressure and pulse rate and the changes in response to treatment also were recorded.

²² Hooker, D. R. *Am J Physiol* **35** 73, 1914, *ibid* **40** 43 (March) 1916.
Hooker, D. R., and Eyster. *Bull Johns Hopkins Hosp* **19** 274, 1908.

Pfann (1904) Sewall¹⁶ (1906), Hooker²³ (1914) and others have shown that the venous pressure has a diurnal as well as other physiologic variations for normal subjects, the usual pressure being from 3 to 11 cm of water. Barach and Marks²⁴ found that in six heart cases only one varied from the normal. Schott²⁵ (1912) found that the greater the insufficiency of the myocardium the greater the rise in venous pressure following exercise.

In the majority of our cases, the arterial pressure was low and the volume and tension of the radial pulse poor. The venous pressure, while it tended to be high, especially during the periods of tricuspid insufficiency, varied a great deal during the course of the individual case and seemed to depend on the state of compensation. The normal varied between 6 and 12 cm of water, with the patient reclining. In marked decompensation with excessive venous congestion and cyanosis, the pressure in two cases rose as high as 22-24 cm of water but usually was in the neighborhood of 15 cm.

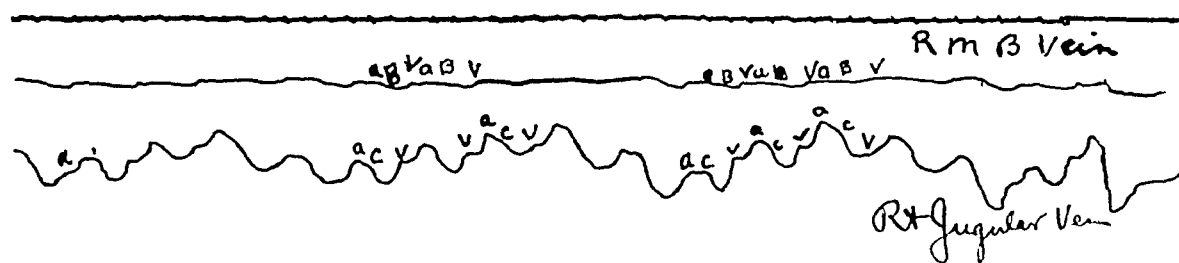


Fig 5 (Case 2) —Right jugular pulse and right median basilic vein immediately after Figure 4 with less swing to the jugular pens

EXPERIMENTAL OBSERVATIONS

We have compiled fifty-six cases, mostly within the period between September, 1921, and May, 1922, all showing pulsations in the basilic veins at some period of their stay in the hospital. Many of these cases have been under observation for a long time. When carefully examined, all these cases have shown a certain degree of myocardial damage and most of the signs and symptoms of the tricuspid syndrome: (1) cyanosis and dyspnea, (2) edema and ascites, (3) dilated right heart to percussion, to fluoroscope and to radiocardiogram, (4) a systolic murmur heard best over the lower part of the sternum, (5) a large, pulsating or tender liver, (6) marked pulsations in the jugular veins of the ventricular type and usually showing a split V wave by polygraph, (7) increased venous pressure (12-16 cm of water), (8) pulsations in the peripheral veins, especially the basilic (both visible and palpable);

23 Hooker (Footnote 22, first reference)

24 Barach, J. H., and Marks, W. L. Arch Int Med **11** 485 (May) 1913

25 Schott. Deutsch Arch f klin Med **107** 375, 1912

(9) myocardial damage, shown by electrocardiograms, and occasionally right-sided hypertrophy. The hearts of some eight of these cases have been examined at necropsy. The tricuspid valves in each case have appeared incompetent and the orifice dilated. All the cases have a clinical diagnosis of chronic myocarditis.

The largest group is the arteriosclerotic division in which there are sixteen cases, three of which had hypertrophied prostates. Eleven of these are known to be dead, three have a poor prognosis, and one is unknown. Rheumatic carditis (showing these pulsations) with twelve cases is next. Three of these are subacute. The remainder have mitral stenosis as their chief lesion. Four of the patients are dead and the remainder have a fair prognosis. There are eleven cases of anemia of some type. Seven are dead, two offer a poor and one a fair prognosis, and the state of one is unknown. There are ten cases of a myocarditis on a toxic basis—eight of these are due to hyperplasia of the thyroid (three of which are dead), one to plumbism and one to obstructive jaundice (dead). The six survivors offer a good prognosis. There are five cases in which syphilis plays the major part—two of the patients are dead and one case in which there is an aneurysm offers a poor prognosis. In the remaining two, the prognosis is only fair. Two other cases offer a good prognosis, one of complete heart block and one of eventration of the diaphragm. Thus, of fifty-six cases there are twenty-eight, or 50 per cent, in which the patients are known to be dead, four offer a poor prognosis, and only eight offer a good prognosis.

PROGNOSIS

In the presence of extensive valvular lesions and marked decompensation, we venture to offer a poor prognosis as the venous pulsations in the veins are usually an indication of pronounced heart failure. In support of this, we have twenty-eight cases in which marked pulsations developed the last few days or weeks before death. All of these cases had a myocarditis and most of them organic valvular lesions as well. Only four cases remain of this group of badly decompensated hearts in which marked venous pulsations were considered an indication for a poor prognosis, and their number is decreasing steadily. Thus thirty-two of fifty cases developing the pulsations presented definitely a very poor prognosis at the time. Of the remainder, the appearance of these pulsations may be considered a warning sign of early heart failure.

The most striking examples are eight cases of marked thyrotoxicosis (from a series of some fifty observed) in which, following suitable treatment of the thyroid, the pulsations disappeared and the patient whose myocardium was not badly damaged completely recovered (five cases). Syphilis, when it is amenable to treatment, falls in the same category. In several patients with decompensated hearts who had shown

pulsations and in whom the pulsations had disappeared under treatment, some extra strain, such as getting out of bed and going to the lavatory, would cause the pulsations to reappear. Along with this the cyanosis and dyspnea increased and a systolic murmur was usually detected where it has been absent just before. Suitable rest then caused the subsidence of symptoms and disappearance of the pulsations in a few hours. Mackenzie states in his chapter on prognosis (p. 348) that "in estimating the value of any abnormal sign or in determining the condition of a heart, the most reliable guide is the manner in which the heart responds to exertion. This again is but an attempt to estimate the amount of reserve force." Usually other clinical signs also warn the clinician of impending danger. The pulsating veins may be, however, the first outstanding sign in the course of the case.

Some twenty-one hospital patients, taken as they came in the wards, and ten normal adults were observed carefully over quite a period of time without showing the pulsations. Only two of these patients had heart disease, and they were well compensated. Acute rheumatic hearts do not show these pulsations as a rule except when they become acutely decompensated. One case followed for four months showed them only twice and then only after the patient got out of bed against orders.

Very often while examining in a routine manner supposed controls, we would find unsuspected signs of early heart failure and the pulsating basilar veins. We have examined within the last three years, since these observations were started, several thousand or so of ambulatory, well compensated clinic patients without having seen the pulsating vein once, yet one or two patients approaching decompensation who show them can usually be found in the clinic or in the ward.

Since the data for these cases was compiled, we have recorded fifty of more cases showing these pulsations.

Observations on the retinal veins suggest that pulsating retinal veins have the same significance as other pulsating peripheral veins.

The following five cases are typical and indicate some of the various conditions in which the pulsations may be found.

REPORT OF CASES

CASE 1—A S., a man, aged 46, was under observation from March 1 to May 26, 1921. The clinical diagnosis was chronic carditis, endocarditis, with mitral stenosis and insufficiency, aortic insufficiency, relative tricuspid insufficiency, chronic myocarditis and chronic pericarditis. There was marked decompensation, cyanosis, dyspnea, orthopnea, edema, ascites, marked venous congestion with pulsating jugular and basilar veins and a large, pulsating, tender liver. There was a large overactive, irregular heart, and it was difficult to differentiate the systolic tricuspid murmur from the loud murmurs heard all over the precordia. The blood pressure was 120 systolic, 90 diastolic, the rate was 70-100 and irregular. The patient was receiving digitalis. The venous pulsations were present off and on during the three months the patient was under observation,

and they were very prominent during the last month just before death. They disappeared with improvement or on relief of the congestion by venous section and returned without fail whenever the patient began to feel dyspneic and uncomfortable again with the breaking of compensation.

April 14, 1921, the pulsations in the basilic veins were marked. Venous collapse occurred at the level of the upper border of the clavicle, approximately 22 cm above the xiphisternum (fifth rib) with the patient in semi-Fowler position. The excursion of the waves at their optimum level was about 2 cm. The venous pressure in the basilic vein with the capsule and mercury surface level with the xiphisternum (fifth rib) averaged 16 mm of mercury (seventeen determinations). This is equivalent to 20.8 cm of water. The blood pressure was 117 systolic, 75 diastolic. The radial pulse was 84.

April 19, 1921, the patient was very dyspneic and cyanotic. Venous collapse occurred at 22-23 cm above the xiphisternum (fifth rib) with the patient in semi-Fowler position. A saline solution manometer was connected to the basilic vein by an intravenous needle. With the vein and surface of the liquid in the manometer level with the manubrium 15 cm above the xiphisternum (fifth rib), there was a rise of 6.4 cm in the saline solution column when the connection was made. Therefore, the venous pressure was equivalent to 21.4 cm of saline solution. Vertical excursions in the manometer column of 0.7 cm occurred with each heart beat. These were registered by a polygraph (Fig. 2). These tracings lacked the small, sharp brachial artery wave shown in the tracings obtained by the capsule on the skin, and may be considered as the true venous pulsations as they seem to the eye and finger. The blood pressure was 120 systolic, 80 diastolic. The radial pulse was 88.

From May 1 to 26, the decompensation was increasing with occasional relief by venesection. The pulsations were visible at all times except after venesection.

A section was made, May 26, six hours after the patient's death. The heart weighed 800 gm., there was typical *cor bovinum*. The right ventricular wall was from 6 to 8 mm and the left ventricular wall from 10 to 12 mm thick. The circumference of the valvular rings was as follows: Aortic, 65 mm; mitral 30 mm, pulmonary 85 mm, tricuspid 130 mm. The chorda tendineae were all thickened and those to the mitral leaflets were very broad (1 cm) and calcareous. The tricuspid valves appeared to be incompetent and the ring relaxed.

The anatomic diagnosis was marked general cardiac hypertrophy and dilatation, chronic mitral and aortic valvulitis with marked stenosis of the mitral valve, less marked of the aortic, fibrosis with calcification of the chorda tendineae to the mitral leaflets with ulceration of the superficial auricular surface and acute thrombus formation, subacute thrombotic endocarditis of both auricles, especially the right, fibrous pericarditis and hydropericardium, general chronic passive congestion, compression atelectasis of the left lung, bronchopneumonia of the right lung. There were multiple areas of fibrosis of the right lung and obliterating pleural adhesions about the mesial surface of the lung on the left, cholelithiasis, fibrous adhesions between the right lobe of the liver and the diaphragm and about the spleen, focal splenic capsular fibrosis, an anemic infarct of the right kidney, slight chronic interstitial nephritis, and multiple erosions of the gastric mucosa.

CASE 2—A man, aged 55, suffered from generalized arteriosclerosis, anginoid attacks, cardiac myomalacia, marked decompensation, with dyspnea, cyanosis, orthopnea, edema and ascites, a large, tender, pulsating liver, and marked pulsations in the jugular and basilic veins and of the dorsal veins of the hands. There was a systolic murmur at the xiphisternum and, questionably, a tricuspid murmur. General improvement was noted under treatment with a disappearance of the pulsations in the basilic veins two days before the patient was discharged at his own request.

The patient returned in an ambulance the following night badly decompensated, with marked venous pulsations and a loud systolic, definitely a tricuspid murmur over the xiphisternum. Direct readings with a venous needle showed a venous pressure of 143 cm of saline solution. The collapsing point of the veins was 17 cm above the junction of the fifth rib with the sternum (patient in semi-Fowler position). The patient developed signs of pulmonary embolism and became markedly dyspneic. Venous engorgement became marked, the blood pressure dropped to 87 systolic, 78 diastolic, while the venous pressure remained somewhat high (15 cm above the xiphisternum). This was relieved somewhat by venous section. The pulsations though faint were observed until death three days later. The venous pressure continued to drop up to the time of death.

The anatomic diagnosis from a section twelve hours later was: Marked disseminated fibrosis of the entire left ventricle and interventricular septum with complete fibrosis at the apex with pocketing, slight coronary sclerosis without occlusion. There were small mural thrombi in the right ventricle and right auricular appendage, one large and several smaller thrombi in the left ventricle—one small pedunculated thrombus. Terminal septicemia was present, marked hypertrophy of the right ventricle, moderate dilatation of both auricles and the right ventricle, marked in the left ventricle, chronic adhesive pericarditis over the right auricle, petechiae over the base of the left ventricle and on the arch of the aorta, small infarcts on the edge of the left lung,

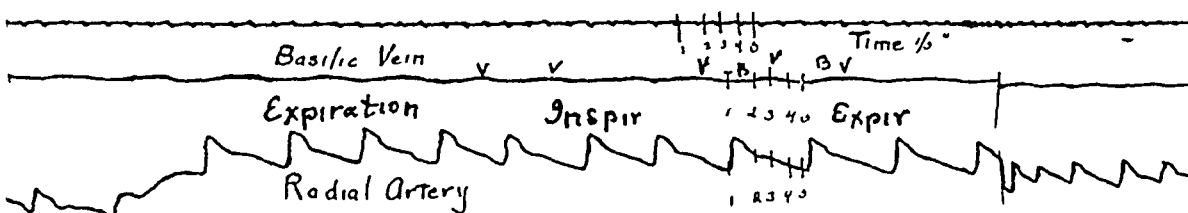


Fig 6 (Case 3)—Tracing of right radial and right median basilic vein with glass capsule in toxic goiter with fibrillation and moderate decompensation. 1 to 2, *B*, brachial and radial artery systole and just before beginning of regurgitant venous wave—visible pulsation in the pen but not easily registered—a little over one-fifth second in length, 2 to 4, main elevation of *V* wave (almost two-fifths second), 4 to 5, end of wave before next systole (almost one-fifth second) and the usual period for the *A* wave, but as in Case 1, the *A* wave is absent so there is no elevation of the venous pulse similar to that in Case 2 (Figs 3-5), 3, apex of wave one-fifth second following dirotic notch (2) and from the beginning of the radial systole, about two-fifths second. It is the venous regurgitant wave or the *V* wave of the jugular pulse.

thrombosis of the large branch of the right pulmonary artery, with large infarcts in the upper and lower lobes of the right lung. There were pneumonic consolidation above the infarct in the lower lobe of the right lung, a light acute fibrinous pleurisy over the infarct in the right lung, a slight pleural effusion, more marked on right, a moderate acute splenic pulp reaction, a moderate chronic diffuse nephritis with a retention cyst in the left kidney, chronic passive congestion of the viscera, and cloudy swelling of the viscera.

The circumferences of the valve orifices were: Aortic 80 mm, mitral 95 mm, pulmonic 75 mm, and tricuspid 140 mm. The tricuspid orifice seemed dilated and the valves incompetent.

CASE 3—A man, aged 48, was under observation with a clinical diagnosis of toxic hyperplasia of the thyroid with toxic myocarditis and auricular fibrillation. The basal metabolism was 84+ per cent. There were marked dyspnea and cyanosis, an enlarged overactive heart, and slight edema of the extremities. The liver was pulsating and slightly enlarged. The jugular and

basilic veins were pulsating markedly. The excursion of the pulsation in the basilic veins was from 2 to 3 cm. A systolic murmur was heard over the xiphoid, and a diagnosis of relative insufficiency of the tricuspid was made. No valvular lesions were present. The systolic blood pressure was 130, the diastolic 80. The pulse rate was 130-100 and irregular.

Polygraph tracings of the jugular vein showed a large undulating jugular wave with an absent A wave, a small C wave and a broad plateau type of V wave almost one-fifth second in duration. The liver tracings (Fig 7) showed a large wave of ventricular type with a prominent and occasionally split V wave. The basilic vein tracings (Fig 6) showed only one large undulating wave, late systolic in time, and following the diastolic notch of the radial pulse. The pen lever registered a small systolic pulsation synchronous with, and starting just an instant before, the radial lever beat. This pulsation was due to the brachial artery nearby and was too faint to show up well on the tracings (Fig 6, B).

Within two weeks following the instillation of radium emanation needles into the thyroid, the pulsations disappeared following the gradual subsidence of all the symptoms relative to myocardial weakness and tricuspid insufficiency. The heart was supported by quinidin and the usual treatment. Six months later, the patient had gained 50 pounds (22.7 kg) and showed no abnormal symptoms.

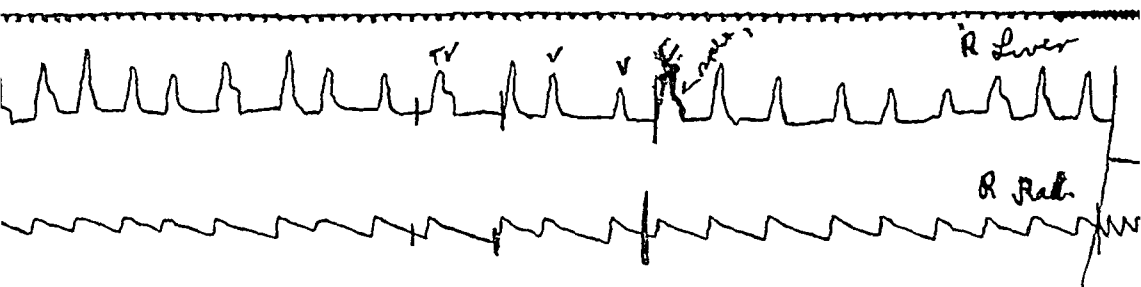


Fig 7 (Case 3)—Liver and radial tracing taken right after the tracing in Figure 6, showing ventricular type of liver tracing with an occasional split V wave somewhat similar to the split V wave in the jugular tracings.

CASE 4—B, a man, aged 34, suffered from combined system disease which was of interest because the venous pulsations led to the early diagnosis of dilatation and failure of the heart in a very spectacular manner.

The lower extremities and sphincters were paralyzed. There was myocarditis of a low grade in keeping with the general weakness and debility. The heart was regular and showed no valvular disease, and seemed able to keep up a good circulation. The patient was being used as a control, showing no evidence of failure, no tricuspid murmur, venous congestion or pulsations in the peripheral veins. Catheterization of about 2 liters of urine brought about an attack of acute syncope. This was followed almost immediately by a period of marked paroxysmal tachycardia (rate, 140-200) for about thirty-six hours. Approximately one hour after the onset of the tachycardia, large pulsations were noted in the basilic veins. A short time later, the liver was found to be tender and pulsating. Edema of the ankles developed rapidly. There was marked cyanosis and dyspnea. The collapsing point of the median basilic veins was 14 cm above the xiphisternum with the patient in a semi-Fowler position. When the pulse rate was slowed down by vagal stimulation, a faint systolic murmur could be heard over the tricuspid area one hour after onset of tachycardia. The right heart was enlarged to percussion. By the next day the pulse rate had dropped to 80. The blood pressure during this time had been 80 systolic, 60 diastolic. The venous pulsations continued over the whole month following the attack up to the last twenty-four hours just

before death, when the venous pressure fell remarkably and the pulsations were no longer visible. The tricuspid murmur, cyanosis and dyspnea, edema and tender liver also persisted. The venous pressure varied considerably—at first it was quite high. The collapsing point was level with the second rib when the patient was in semi-Fowler position, or approximately 15 cm above the xiphisternum. Following the attack of syncope, it dropped quite markedly (from 5 to 10 cm) and varied from time to time. The pulsations also varied in the prominence and amount of excursion. With a high venous pressure they were generally pronounced and vice versa. No necropsy was permitted.

CASE 5—A man, aged 45, had a history of rheumatic fever. The clinical diagnosis was chronic myocarditis and endocarditis with mitral and tricuspid stenosis and insufficiency, auricular fibrillation and decompensation with marked anasarca.

April 4, 1922, the heart was dilated to the right and the left with a coarse vibratory systolic murmur loudest over the end of the sternum and merging with a systolic murmur over the apex of different quality and intensity. There was a large, nontender, pulsating liver and marked pulsation of the veins over the forehead, arms, hands and feet was noted. Pulsations were systolic or presystolic in time over the veins and the liver. There was a marked bounding pulsation in the jugular veins, the right jugular bulb especially standing out prominently about 2 cm in diameter, in the right supraclavicular space. The systolic blood pressure ranged from 120 to 100, the diastolic was 78. The pulse was 50-110. A polygraph tracing of the jugular vein showed an absent A wave and a high split plateau type of V wave. The venous pressure was approximately 14 cm above the xiphisternum with the patient in a semi-Fowler position. Pulsations could not be recorded from the other veins as the polygraph was out of order.

Under digitalization and later quinin the decompensation improved and the venous pressure and pulsations diminished, but these were still present on discharge three weeks later.

COMMENT

The tricuspid syndrome has been discussed by many authors, usually with entire agreement as to what actually composes a relative insufficiency of the tricuspid valve, so that we shall not review it here. Its signs and symptoms are briefly enumerated above. The common occurrence of relative insufficiency is also well recognized. Leube²⁶ (1905) says, "Das eine funktionelle Trikuspidalinsuffizienz in Verlaufe der Anämie auftreten kann, ist selbst verstehtlich." G. Canby Robinson²⁷ (1920) writes that "an increase in pressure within the right ventricle sufficient to produce tricuspid incompetence may be caused by asphyxia or even by prolonged holding of the breath, or by severe exertion. Lesions such as emphysema or fibrosis of the lungs, nutritional disturbances in the cardiac muscle, such as occur in severe anemia, in fever, or with a diminished blood supply to the myocardium resulting from obstruction in the coronary arteries" give rise to incompetence. Our series of cases presents all of these factors.

Mackenzie¹ states that "although actual disease of the valves is rare, incompetence of the tricuspid orifice is extremely common, so common,

26 Leube Ztschr f klin Med 57 199-207, 1905

27 Robinson, G C Nelson's Loose-Leaf Living Medicine 4 376, 1920

indeed, that I am inclined to look upon the valves as being barely able to close the orifice perfectly Experimentally, it has been found impossible to raise the pressure in the right ventricle, on account of the ease with which regurgitation takes place through the tricuspid orifice " He has noted the frequent occurrence of a "greatly widened orifice (tricuspid) postmortem, while during life there was no systolic murmur " This seems to be the usual experience of most pathologists, in fact, the dilatation of the tricuspid ring and incompetency of its valves is looked on with complacency and even regarded as "normal" at postmortem

We wish to emphasize, therefore, a certain element in myocardial disease which plays the great part in causing the pulsations in the veins, and that is the weakness of the tricuspid orifice King⁶ (1837) and Gibson²⁸ (1880) thought that this weakness served a safety valve function in relieving the overburdened ventricle

The pressure on the right side of the heart is low and the musculature thin and any increase in pressure or stasis from weakness, etc. may cause dilatation very readily because of the thin and flabby tissues involved Myocardial weakness is the fundamental factor for the onset of decompensation and the occurrence of dilatation

Benson⁵ in 1836 contended that the pulsations in the peripheral veins were due to the propagation of a regurgitant impulse from the right ventricle through the auriculoventricular orifice into and throughout the distended veins, no matter how incompetent their valves

We think it can really and conclusively be demonstrated in several ways that these pulsations are centrifugal, associated with venous stasis and propagated from the right ventricle when that chamber contracts:

First, if the basilic vein is just obliterated several centimeters proximal to its branches, by very light pressure made with the thumb of the hand supporting the patient's arm at the elbow, the pulsations can be made to disappear and reappear at will It will be noted on close observation at these times that the pulsations are distinctly centrifugal. They do not occur in the distal veins while the veins fill up during the period of compression of the basilic vein

Second, if the forearm is held perpendicularly and the basilic vein is again lightly compressed, during the rise of the venous level due to the blood flowing into the veins from the capillaries there is no observable pulsation (the explanation suggested by Gaertner among others) If the level of compression on the basilic vein is now raised above the "critical point" of collapse the pulsations will be seen proximal to the point of compression and not distal to it A similar maneuver

28 Gibson Edinburgh M J 25 979, 1880

has been used on the jugular vein by many authors to demonstrate tricuspid insufficiency

Third, if pressure is made on the midportion of the veins and the proximal portion is stripped toward the heart, the pulsations return and mount higher and higher peripherally in waves synchronous with the heart beat to the critical level or to the compression point

It has often been observed that pressure on the liver will make the pulsations in the jugular veins more prominent. We have found that firm pressure on the liver will raise the collapsing point in the basilic vein several centimeters (from 6 to 10 cm), depending on the degree of pressure. This gradually declines in from five to ten seconds, as the heart and vessels compensate for the increased congestion even though the pressure is continued. The respiratory variations are seen to occur as stated by Gaetner and others. We have been unable to observe these pulsations in several experiments on ourselves following the relaxation of the capillaries by heat.

Reference has been made in the literature to pulsations seen in the veins of the arm, forearm and hand, the femoral or crural veins, the veins of the lower leg and foot, varicose veins of the leg, the veins of the thorax and abdomen, the forehead, temples and scalp, the retina and the thyroid. Of course, the jugular veins throughout their course have received much study and the interpretation of their pulsations has given rise to a tremendous literature. Pulsations elsewhere than in the neck and basilic veins, veins of the hand and the retinal vessels seem to be uncommon in our experience, except in cases of marked decompensation with pronounced regurgitation and engorgement. In all such cases the prognosis was very bad as the patients died soon after. In this they resembled the cases reported in the literature. In our cases, as noted by the early workers, venous section caused the pulsations to disappear because congestion was relieved.

Mackenzie²⁹ (p. 25) warns us that "it must be borne in mind that between the right ventricle and the jugular vein there is interposed a dilating auricle and a capacious superior vena cava and that a wave can only appear in the jugular after the auricle and vena cava have been overfilled." It would appear that a small volume of blood regurgitated into the congested vena cava would not make much difference in the pulsations on the surface of a large vein like the jugular vein. In the case of a small vein, however, in the presence of congestive failure, this would have a more evident influence on the excursions of its surface because of the relatively greater volume changes per caliber. In the very small veins the walls become less flaccid as the caliber decreases, so that the variations in surface level may be present although they are not

visible This is also true when the vein walls are sclerotic, as is often the case of the dorsal veins of the hands in elderly people

Anatomically, the liver, the neck and axillary veins are so situated as to receive directly the regurgitant wave coming from the right ventricle through a dilated right auricle into the vena cava The liver can readily absorb small pulsations because of its structure, and they may not be noted The jugular vein, because of its height above the heart, is not affected so much by a relatively small increase in venous pressure and congestion, and because of its large size it readily absorbs small changes from regurgitation which may escape observation These may be detected, however, by such changes as splitting of the V wave in the polygraph tracings³⁰

Gaertner suggested that in the manner of a manometer the arm veins would show the venous pressure, and that this could be considered as equal to the auricular pressure These veins are of relatively good caliber and their connections with the engorged vena cava and right auricle are quite simple and direct If congestion occurs with the consequent elevation of the venous pressure, dilatation not only of the auriculoventricular ring becomes possible but also dilatation of the much more elastic veins is readily demonstrable in the periphery Benson's⁵ argument that a pulsation can readily travel up a vein already distended by congestion without interference by its valves seems well taken It is very likely, furthermore, that as the congestion (and the venous pressure) becomes greater, the venous valves both of the jugular bulb and further in the periphery become incompetent and the regurgitant waves can progress distally in greater volume This seems borne out by the variation in size of the venous pulsations in proportion to the changes in venous pressure and congestion It is only logical, therefore, to suppose that a regurgitation of only a small amount of blood from the right ventricle would cause visible oscillations of some magnitude in these veins It is for these reasons and on our experimental observations that we base our contention that pulsations in the basilic veins are early associated with venous stasis and tricuspid insufficiency³¹

30 Mackenzie (Footnote 28) Lewis Clinical Disorders of the Heart Beat, Ed 5, Shaw and Sons, 1920

31 Since these observations have been made, two unusual cases have presented some interesting pulsations A patient with marked organic tricuspid stenosis and insufficiency, proved by necropsy, showed marked presystolic and systolic pulsations in the liver, jugular veins and vessels of the arm, hand and retinal veins Another patient with complete heart block showed pulsations in the basilic vein with the contraction of the auricle and superimposed pulsations following the contraction of the ventricles There were long periods of asystole of the ventricles with well marked peripheral vein pulsations associated with contraction of the auricles Attacks of ventricular tachycardia also were observed with pulsations of the veins following contractions of the auricles and ventricles superimposed

It seems that the prophecy made by King in 1837 is being verified. He said

I may venture to express an anticipation that more careful observation, even with the unassisted eye, will detect pulsating veins more frequently than has hitherto been done, and even render this fact available in diagnosis. I would also hope that the examination of the veins by the help of the sphygmometer may become still more generally serviceable in practical matters.

SUMMARY

The literature contains many isolated references to venous pulsations in the peripheral veins, most of which were published many years ago. The significance of these pulsations has not been generally recognized. It is our opinion, based on careful study of over fifty-six cases showing these pulsations, that they are present only during congestive failure and relative incompetency of the tricuspid valve. These observations have been checked up on several thousand clinic and ward patients seen within the last three years and fifty more cases noted with tricuspid syndrome and pulsations in the basilic veins. Their presence in a large variety of ward cases suggests that they are of value in making an early diagnosis of myocardial weakness or failure with passive congestion and relative tricuspid insufficiency in cases in which these factors would ordinarily be overlooked. Their magnitude seems to be an indication of the degree of incompetency of the tricuspid valve and also of the heart musculature. In cases of markedly damaged myocardium, their presence is thought to offer a bad prognosis, as twenty-eight out of thirty-two such patients have died within a year after observation. Cases featuring hyperthyroidism, arteriosclerosis, anemia, mitral stenosis and syphilis are the most common in presenting these pulsations.

The peripheral pulsations are more prominent and more frequently observed in the basilic veins of the arm and forearm. Their pulsations follow the brachial systole. This retardation of the regurgitant stasis waves seems to be due to the relaxed and engorged state of the veins and a decreased velocity in a low pressure system and is similar to the A, C and V waves of the jugular pulse. The congestion allows the propagation of the impulse even though the valves may be competent.

CONCLUSION

Pulsations in the peripheral veins and especially in the basilic veins of the arms are suggested as an early and valuable sign in detecting the presence of myocardial or congestive heart failure together with a relative tricuspid insufficiency. Their magnitude interprets the degree of myocardial and tricuspid incompetency. Their appearance in cases of marked decompensation suggests a bad prognosis.

THE EFFECT OF CHANGES IN POSITION OF THE HEART ON THE Q-R-S COMPLEX OF THE ELECTROCARDIOGRAM

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That the position of the heart in the chest has an influence on the shape of the electrocardiogram as taken by the usual three leads was pointed out in the early work from Einthoven's laboratory¹ The modifications appearing on rotation of the body from side to side, on changes from the recumbent to the erect position, and in the phases of respiration have all been attributed to this factor Dextrocardia offers one of the greatest possible variations in cardiac position and here, as is well known, the electrocardiogram is reversed Stout subjects with transversely placed hearts usually give electrocardiograms with R₃ of least amplitude while slender subjects with pendulous hearts usually show the least amplitude in R₁ Lateral displacements of the heart due to pleural effusions, air or adhesions also are known to modify the electrocardiogram According to Pardee,² such displacements to the right reduce the height of R and bring Q into prominence Movement of the heart to the left, on the contrary, changes the curves but little

In man experimental procedures designed to alter the heart-thorax relationships other than those mentioned have been obviously impossible Several workers have, however, varied the position of the leads so that there was at least a clever imitation of a change in the heart position Herrmann and Wilson³ substituted Leads II and III for Leads I and II and inverted Lead I By repeating this process they practically rotated the heart in stages of 60 degrees each Counterclockwise rotation gave curves resembling left sided ventricular preponderance, while clockwise rotation gave curves resembling right sided preponderance Cohn⁴ rotated his leads around a triangle laid out as large as possible on the chest This simulated the rotation of the heart on its antero-posterior axis Many of the pictures secured resembled preponderance

*From the Physiological Laboratory of the University of Wisconsin Medical School

1 Einthoven, Fahr and de Waart Arch f d ges Physiol **150** 275, 1913

2 Pardee Clinical Aspects of the Electrocardiogram, New York, Paul B Hoeber, 1924, p 45

3 Herrmann, G R, and Wilson, F N Heart **9** 91 (April) 1922

4 Cohn Heart **9** 311 1922

curves at least so far as the Q-R-S complex was concerned. The T waves, however, were always in the same direction as the main deflection.

All the authors mentioned above have agreed that the position of the heart in the thorax might greatly influence the electrocardiogram and that at times this might be far reaching. For example, in the case of a left ventricular preponderance, the condition as shown by the curves might be estimated as marked, slight, or even absent according to whether the heart was in a transverse or pendulous position. It is thus readily seen that an accurate analysis of the effects of cardiac position on the electrocardiogram would be of value not only in interpreting variations in normal records but in evaluating abnormal curves.

Animal experimentation has not been used to any extent in this problem owing to the general feeling that manipulation with the chest open made it impossible to secure electrocardiograms sufficiently normal to be of value. So far as we can find the only paper dealing with the problem in animals is that of Boden and Neukirch,⁵ who led off from a vessel of saline solution in which an isolated perfused heart was placed. With such a device the heart could be placed in any position desired relative to the leads. They found that rotation of the isolated human or animal heart around its longitudinal axis to the right gave a left sided effect, while rotation in the opposite direction gave curves resembling right sided preponderance. Their experiments, being made under such highly artificial conditions, have not seemed very convincing to other workers.³

In the work here reported we have secured rotation of the heart around its anterior-posterior and longitudinal axes. Dogs under morphin-ether anesthesia were used in all experiments. In the first series of experiments, a rib was resected and ligatures were tied around the pericardium just below the apex of the heart. The attachments of the pericardium to the diaphragm were broken up, and one ligature was carried by a needle through each lateral wall of the thorax. By pulling on these ligatures the apex of the heart could be brought in contact with either side of the thorax, the heart thus describing an arc of about 60 degrees on its anterior-posterior axis. The opening in the chest was closed and the animal resumed its own respiration. Electrocardiograms were then made with the heart in various degrees of rotation.

In later experiments the chest was split in the midline and the animal kept throughout under artificial respiration. The more complete exposure afforded in these cases allowed us, by the use of small fish hooks inserted either into the pericardium or through the pericardium

⁵ Boden and Neukirch. *Arch f d ges Physiol* **171** 146, 1918

into the epicardium, to rotate the heart on its longitudinal axis. Ligatures placed as before brought about rotation from side to side. The heart was anchored at its base by two ligatures sewed into the fascia around the aorta and fastened to the thoracic walls.

The majority of our records were made by taking simultaneously with two Edelmann galvanometers, first Leads I and II and then Leads II and III. In a few recent experiments we have added a third galvanom-

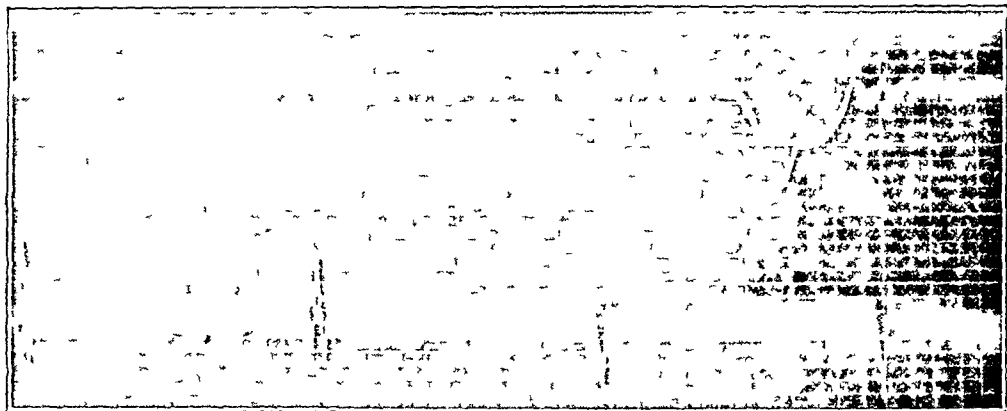


Fig 1—Increase of Q wave and disappearance of S wave in Lead I on pulling the heart to the right

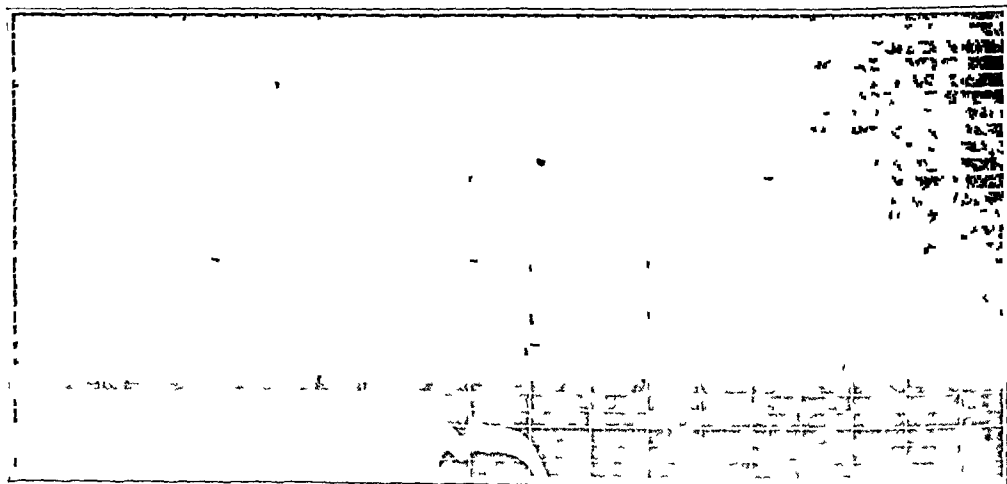


Fig 2—Disappearance of the Q wave in Lead I on pulling the heart to the left

eter and taken all three leads simultaneously. Throughout our work we have accepted the validity of the Einthoven triangle, and have used his formula in determining all electrical angles. Our standard has been that R_1 and R_3 should approximately equal R_2 . We have allowed one-tenth millivolt variation in these measurements, since it was impossible under the conditions of our experiments exactly to reproduce the same degree of rotation for each set of records. This variation if attributed entirely either to R_1 or R_3 would produce a variation of not

more than 5 degrees in the electrical angles. This necessary error in technic is not large enough to interfere with a determination of the general effect of a given rotation. In those cases in which R_1 and R_3 failed to equal R_2 by more than a millimeter the records have still been found valuable. By using, first, the formula of Einthoven which determines the electrical angle from R_2 and R_3 and, second, the formula involving R_2 and R_1 , the greatest variations in the electrical angle which are possible from the given data are found. If these point in the same direction, the experiment is of course satisfactory and indicates the qualitative effect of the rotation. Since phase differences might lead to uncertain calculations, R_2 has always been used as the standard for measurements. R_2 and R_3 are usually synchronous, the variations in synchrony being found most often between R_1 and R_3 .

In those experiments in which the chest was opened, ligatures applied and the thorax closed, the electrocardiograms were usually indistinguishable from controls taken before the operation. Electrocardiograms from animals with an open chest are also usually normal. By this we mean that the waves are all apparent and of the usual form. Only two variations are common. R_3 is often notched and the T wave is apt to be negative or diphasic, a condition which may appear in the dog under almost any operative procedure.

ROTATION AROUND THE ANTERIOR-POSTERIOR AXIS TO THE RIGHT

The axis for rotation around the anterior-posterior axis to the right was where the base of the heart crossed the midlongitudinal line. This point was chosen because it was here that the heart was anchored by ligatures. A line was drawn from this point to the apex of the heart. The angle between this line and the horizontal has been termed the anatomic angle. This anatomic angle as determined in the open chest was ordinarily about 80 degrees. When pulled to the right it was increased to approximately 110 degrees, and when pulled to the left it was reduced to about 60 degrees. According to the simple application of Einthoven's triangle, when the heart is pulled this distance to the right one would expect a decreased or inverted R_1 , a decreased R_2 and an increased R_3 . An analysis of our results, however, shows that R_3 in twenty-nine experiments increased in only 76 per cent of the cases, R_2 decreased in only 31 per cent and R_1 decreased in only 45 per cent. From this it is evident that the electrical axis did not regularly vary with the anatomic, and that in some cases it increased and in others decreased. It varied in direction inversely with the increase or decrease in size of R_1 .

In Table 1 data are presented illustrating these conditions in six experiments. The other twenty-three experiments showed similar variations.

The most consistent change in these curves concerned the Q wave. In sixteen of the twenty-nine cases the descent of the Q increased in Lead I with rotation to the right. In ten others Q appeared after being absent in the normal. In three cases Q was absent or remained the same under both conditions. It is to be recalled that Q is seldom if ever found in Lead I in right sided preponderance but that it may appear in Lead III. Yet in our attempts to imitate right sided preponderance by rotation to the right around the anterior-posterior axis, the Q wave either appeared or increased in Lead I in all but three of twenty-nine cases. Figure 1 gives an illustration of such a change in the Q wave. In Lead II the Q wave appeared in 80 per cent of the curves, an increase of 28 per cent over the normal, while in Lead III the Q wave was found in only 34 per cent of all records, a decrease of 11 per cent from normal. This indicates that we were not very

TABLE 1—*Effect of Rotating the Heart to the Right Around Its Anterior-Posterior Axis*

	Experi- ment	Anatomic Axis, Degrees	Electrical Axis, Degrees	R ₁	R ₂	R ₃
Normal	6	83	67	7.0	20.0	12.0
Pulled right		110	63	12.0	27.3	15.0
Normal	18	80	83	1.0	8.5	7.4
Pulled right		108	14	4.0	3.0	-1.2
Normal	8	78	70	8.0	23.0	15.0
Pulled right		98	75	7.0	27.0	20.0
Normal	16	93	68	5.0	15.9	10.0
Pulled right		115	86	1.25	19.5	18.0
Normal	10	74	71	4.0	12.0	8.0
Pulled right		113	65	4.0	10.0	5.8
Normal	4	72	81	0.5	12.0	10.0
Pulled right		100	109	-2.0	4.0	6.0

closely imitating right sided hypertrophy. These results are, however, in line with what Boden and Neukirch found on rotation to the right around the longitudinal axis.

On rotation to the right around the anterior-posterior axis, the S wave behaved in a manner exactly opposite that of the Q wave, and therefore opposite to what should be expected were we imitating right sided preponderance. When the heart was pulled to the right, the S wave disappeared in Lead I in seven cases in which it had been present normally, increased in only one, and appeared anew in only one. Figure 1 illustrates such a disappearance of the S wave. Yet it is to be remembered that right sided preponderance is characterized by a large S in Lead I. S in Lead III was present in all cases after right sided rotation, having increased or appeared anew in four experiments. In right sided preponderance S₂ is of course small or abstract.

It was evident from the foregoing data that merely pulling the heart to the right did not give the picture expected on theoretical grounds, nor did it resemble right sided preponderance to any great degree

ROTATION AROUND THE ANTERIOR-POSTERIOR AXIS TO THE LEFT

The axis for rotation around the anterior-posterior axis to the left was the same as for the right, the heart being pulled to the left until the apex touched the chest wall, decreasing the anatomic angle to about 60 degrees. According to the principle of Einthoven's triangle, one should expect such a rotation to the left to give an increased R_1 , a variable or increased R_2 , and a small or inverted R_3 . Our results show that R_1 increased in 63 per cent of thirty experiments, R_2 decreased in

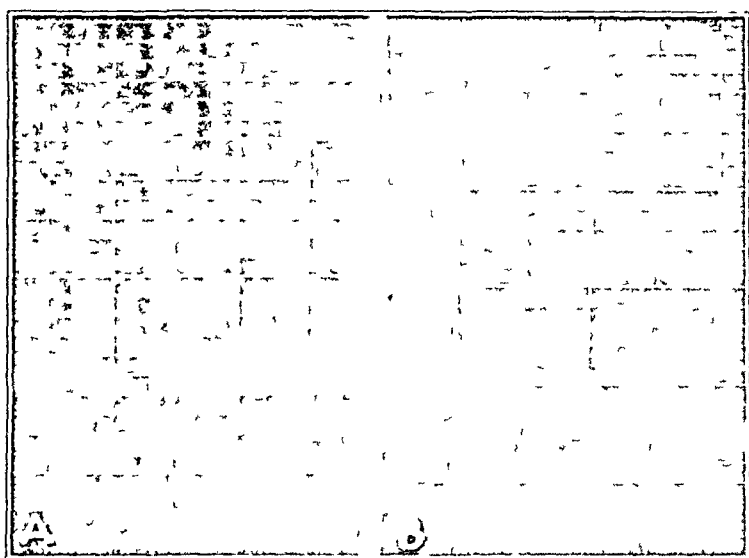


Fig 3—Appearance of the Q wave and the disappearance of the S wave in Lead III on pulling the heart to the left, A, before, and B, after heart is pulled to the left

60 per cent, and R_3 decreased in 80 per cent. This is a much closer approximation to the theoretical than is secured on rotation to the right, and the electrical angle is accordingly reduced in the majority of these experiments, in some of them it follows the anatomic axis rather closely. Many of the curves very definitely resemble left sided preponderance. The exceptions, however, are sufficiently numerous to indicate that, as before, some other factor was operating in these rotations. Table 2 shows data from six experiments in which the heart was rotated to the left. The other experiments showed similar variations and are therefore not tabulated.

On rotation to the left the occurrence of the Q wave is reduced from 62 per cent in Lead I to 15 per cent, while in Lead III it increases from 45 to 68 per cent. Figures 2 and 3 illustrate these conditions

This is, of course, exactly the opposite to what is found in left sided preponderance, in which condition Q may be found in Lead I but is rarely if ever found in Lead III

The S wave again shows a direction of change opposite to that of Q in all leads. It appears less often in Lead III than in the normal, but more often in Lead I. The percentage of change, however, is not so large. This again is exactly the opposite to the average results for left sided preponderance. Figure 4 illustrates the appearance of S in Lead I.

ROTATION AROUND THE ANTERIOR-POSTERIOR AXIS TO THE RIGHT
WITH COUNTERROTATION AROUND THE LONGITUDINAL
AXIS TO THE LEFT

The results so far presented show that rotation around the antero-posterior axis neither consistently fulfils the requirements of Einthoven's

TABLE 2—*Effects of Rotating the Heart to the Left Around Its Anterior-Posterior Axis*

	Experi- ment	Anatomic Axis, Degrees	Electrical Axis, Degrees	R ₁	R ₂	R ₃
Normal	11	76	80	27	11.5	90
Pulled left		51	82	16	11.7	100
Normal	10	74	71	40	12.0	80
Pulled left		48	49	100	15.0	50
Normal	15	85	77	58	27.5	21.1
Pulled left		72	68	95	28.2	17.7
Normal	12	77	57	65	13.0	60
Pulled left		64	40	105	13.0	20
Normal	27	83	60	42	8.3	42
Pulled left		52	83	10	8.3	72
Normal	22	77	65	53	12.0	69
Pulled left		67	43	70	9.0	22

triangle nor duplicates the usual results found in ventricular preponderance. It was obvious that another factor was involved. This we found to be rotation around the longitudinal axis of the heart, it being impossible to displace the heart to either side without such rotation. An anatomic examination showed that our right displacements involved a rotation around the longitudinal axis of about 24 degrees. Displacement to the left occasioned a longitudinal rotation of about 17 degrees. It seemed that the smaller degree of rotation in this direction might account for the closer approximation to the theoretical.

We next ran a series of experiments in which the heart was first displaced to the right and then counterrotated on the longitudinal axis to the left in the hopes of compensating for the rotation around the longitudinal axis involved in the displacement. Data from six such experiments are presented in Table 3. It will be noted that, regardless of whether the electrical angle increased or decreased on mere displacement,

it consistently increased above normal on the counterrotation. This never failed in twelve experiments. Although in all these cases on counterrotation the electrical axis approached the anatomic it equaled it in only one case. This was due to two reasons, one being that what we have called the anatomic axis is a line from the point of rotation to the cardiac apex. It is from 10 to 15 per cent steeper than the axis described by Cohn⁴. The other is that the exact extent of counterrotation necessary could of course not be determined at the time of experiment but had to be estimated at necropsy.

TABLE 3—*Effects of Rotation to the Right Around the Anterior-Posterior Axis Followed by Counterrotation to the Left Around the Longitudinal Axis*

	Experi- ment	Anatomic Axis, Degrees	Electrical Axis, Degrees	R ₁	R ₂	R ₃
Normal	27	83	67	3	6.5	4
Pulled right		120	53	6	9	3.5
Counterrotated left		120	93	0	7.5	8
Normal	24	84	63	8.9	20	10.9
Pulled right		112	72	5.5	17	11.8
Counterrotated left		112	{83 }85	2.2	{16 }12	10.9
Normal	28	82	51	7.3	11.1	4
Pulled right		108	74	3.2	11.1	8
Counterrotated left		108	90	0	10	10
Normal	21	80	67	6.4	17.3	10.5
Pulled right		110	97	-1.1	12.7	14.7
Counterrotated left		110	113	-2.2	10	13.1
Normal	33	80	84	2.0	12.6	11.1
Pulled right		105	64	5.0	14.7	8.3
Counterrotated left		105	86	1.5	8.4	7.8
Normal	31	80	68	5.0	2.0	0.0
Pulled right		115	81	19.0	18.0	18.0
		115	87	12.0	15.0	17.0

In all these experiments R₁ decreased and R₃ increased relative to R₂. This of course is necessary if an increase is to be found in the electrical angle. Curves illustrating this point may be seen in Figure 5, A. As compared with the curves obtained from displacement to the right, on counterrotation the Q wave in Lead I disappeared in 27 per cent of the cases and decreased in 50 per cent. In Lead III the Q wave, however, increased to 80 per cent of all cases. The S wave was only occasionally present in Lead I but was found in all cases in Lead III. From this description it is evident that cardiac displacement to the right with counterrotation on the longitudinal axis to the left gives definite signs of right sided preponderance. These are to be seen in the R and Q waves. The S wave does not follow the rule for right sided preponderance.

ROTATION AROUND THE ANTEROPOSTERIOR AXIS TO THE LEFT
WITH COUNTERROTATION AROUND THE LONGITUDINAL
AXIS TO THE RIGHT

In twelve experiments, with two exceptions, the electrical angle was greatly decreased on counterrotation, regardless of the direction of the change on mere displacement to the left. The two exceptions may well have been examples of overcompensation. Table 4 gives the data from five of these records. R_1 is always increased and R_3 decreased in relation to R_2 . In addition R_2 itself always showed a decrease on counterrotation. On counterrotation the Q wave in Lead I increased in occurrence from 15 per cent in the simple displacement curves up to 70 per cent. In Lead III it decreased from 68 to 30 per cent. The S

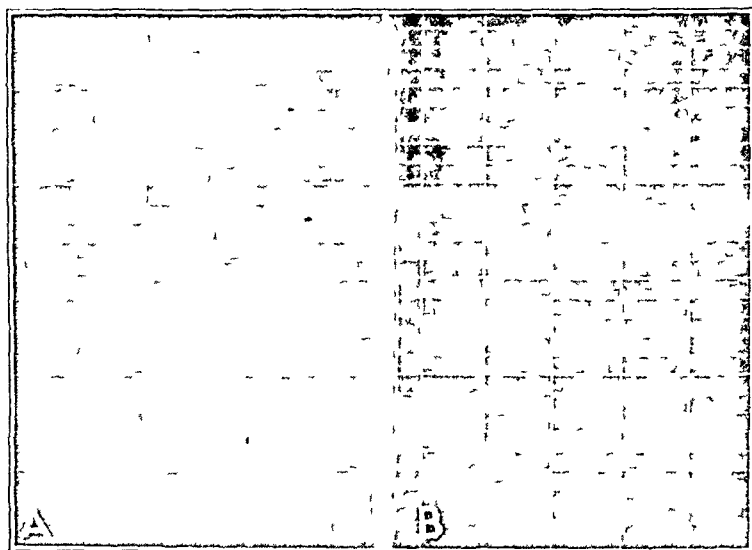


Fig 4—Appearance of S wave in Lead I on pulling the heart to the left, A, before, and B, after the heart is pulled to the left

wave was present in all cases in Lead III after counterrotation, and in the majority of cases it was considerably deepened.

From the foregoing it will be seen that displacement to the left with counterrotation gives a picture practically identical with left sided preponderance. Many of the curves are very characteristic indeed, as may be seen in Figure 5, B.

HEART HELD IN ITS NORMAL POSITION AND ROTATED AROUND ITS
LONGITUDINAL AXIS TO RIGHT AND LEFT

From the preceding experiments it seemed evident that right and left sided displacements around the anterior-posterior axis did not give the expected results owing to the simultaneous rotation which necessarily occurred around the longitudinal axis. This point was definitely proved

by holding the heart in its normal position and rotating either to the right or left around the longitudinal axis. In Table 5 may be seen the result of such rotations.

In all cases tried with rotation to the right, the electrical angle was remarkably decreased, several times becoming negative, this being due to increases in R_1 and decreases in R_3 . The Q wave in Lead I, increased both in incidence and size, was present in all cases on rotation. It was invariably absent in Lead III. The S wave decreased in Lead I, but was found in all cases in Lead III. These are typical signs of left sided preponderance. Curves illustrating rotation to the right may be seen in Figure 5, C.

In Table 5 are also presented the data for rotation to the left. In all cases the electrical angle was increased owing to a smaller R_1 and

TABLE 4—Effects of Rotating to the Left Around the Anterior-Posterior Axis Followed by Counterrotation Around the Longitudinal Axis to the Right

	Experiment	Anatomic Axis, Degrees	Electrical Axis, Degrees	R_1	R_2	R_3
Normal	23	86	46	8.7	12	3.5
Pulled left		67	30	10	10	0
Counterrotated right		67	22	6.9	6	-1
Normal	25	78	69	2.5	9	5.8
Pulled left		61	45	5.8	8	
			65		7	
Counterrotated right		61	-14	10.8	3	-4.1
			-5			
Normal	28	82	51	6	9.4	4
Pulled left		64	59	4	7.8	4.5
Counterrotated right		64	13	8	5.6	-2
Normal	30	82	57	9.1	16.2	7.5
Pulled left		67	54	7.7	13.7	5.6
Counterrotated right		67	30	10	9.4	0
Normal	22	77	65	5.2	12.0	6.9
Pulled left		67	43	7.0	9.0	2.2
Counterrotated right		67	30	7.0	8.0	0.0

a larger R_3 . Q decreased very markedly both in size and incidence in Lead I but increased also markedly in Lead III. The S wave on the contrary increased in Lead I and decreased in Lead III. These are the signs of right sided preponderance. Curves illustrating rotation to the left may be seen in Figure 5, D.

COMMENT

Our results do not seem to agree with Einthoven's explanation for the rather frequent exaggeration of S in Lead I when the subject turns on his right side. Einthoven¹ suggested that the appearance of this wave was due to the projection of a sagittal potential on the frontal plane due to a rotation around the longitudinal axis. One would expect such a rotation to occur from left to right, but experimentally this rotation has always given us an enlarged Q and a reduced or absent S in Lead I.

Herrmann and Wilson's³ conclusion that there is no definite relation between the form of the ventricular complex and the relative weight of the two ventricles unless there is a marked hypertrophy receives both justification and explanation by our findings. Within certain limits the signs of ventricular preponderance may be accentuated, neutralized or reversed by varying the position of the heart, particularly the degree of rotation around the longitudinal axis. These effects are, of course, most marked when the signs of preponderance are least marked, that is, in cases of slight hypertrophy. When the heart is greatly hypertrophied, no change in position is sufficient to obscure the true picture.

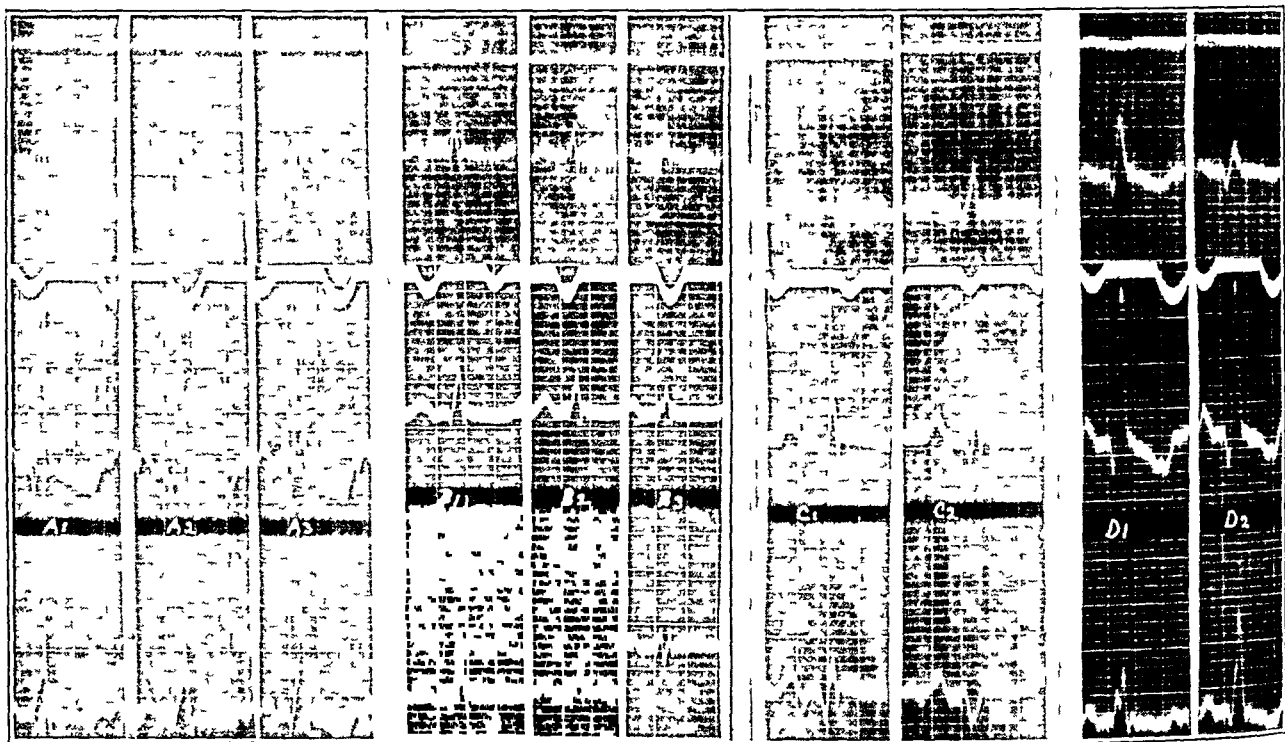


Fig 5—In these records the three leads were taken simultaneously. Lead I, the upper curve, was taken on one camera, while Leads II and III, the middle and lowest curves, were recorded together on another. Before the two slits a horizontal wire was raised and lowered at a rapid rate so that the vertical lines have no time value but they do mark synchronous points. Time is recorded between Leads II and III in one-fifth second. A1, normal, A2, heart pulled to the right, A3, heart pulled to the right and rotated to the left on the longitudinal axis, B1, normal, B2, heart pulled to the left, B3, heart pulled to the left and rotated to the right on its longitudinal axis, C1, normal, C2, heart rotated to the left on its longitudinal axis, D1, normal, and D2, heart rotated to the right on its longitudinal axis.

It would be of great interest and value to know just what degree of left or right sided hypertrophy could be obscured by the rotations involved in the shift of position occurring when the subject lies on the left or right side. Such an investigation would however involve the statistical

treatment of electrocardiograms in the various positions, roentgen-ray shadows of the heart, and a comparison of the ventricular weights secured at necropsy

A recent paper by Dieuaide⁶ is of interest in this connection. Dieuaide proposes diagnosing adhesive pericardial mediastinitis by the absence of variations in the electrocardiogram when the subject changes his position from side to side. Judging from our data it would be expected that no patient with adhesive pericardial mediastinitis would show variations due to change of body position if the adhesions were sufficient to anchor the heart, but on the other hand all patients failing to show variations with a shift of position could hardly be expected to have mediastinitis, the reason being that when the patient is turned to either side, the rotation of the heart around the longitudinal axis may exactly neutralize the effect of the rotation around the anterior-posterior

TABLE 5—*Rotation to the Right and Left Around the Longitudinal Axis Only*

	Experiment	Electrical Axis, Degrees	R ₁	R ₂	R ₃
Normal	22	64	25	58	33
Rotated right		—7	60	21	—33
Normal	25	68	33	80	50
Rotated right		—6	96	40	—58
Normal	28	63	55	100	55
Rotated right		16	85	67	—22
Normal	26	60	45	100	50
Rotated left		81	30	133	110
Normal	28	62	46	106	56
Rotated left		106	—31	75	106
Normal	30	53	78	139	56
Rotated left		72	44	122	83

axis, and the result in the electrocardiogram will be the same as if no movement has occurred

The results obtained in our experiments with rotation around the longitudinal axis seem to bear quite directly on the theory of Groedel and Monckeberg⁷ as to the cause of hypertrophy curves obtained in various diseases of the heart. These workers found an inverted R in Lead I in such diseases as pulmonary insufficiency, pulmonary stenosis, open ductus arteriosus, tricuspid insufficiency, and in some mitral stenosis cases. In all of these diseases, it has been found at necropsy that the right ventricle hypertrophies, not by extending to the right, since it would then have to push aside the right auricle which is fixed by the venae cavae, but by rotating slightly around the axis of the cavae and then by extending up and to the left in the direction of the

6 Dieuaide, F. R. The Electrocardiogram as an Aid in the Diagnosis of Adhesive Pericardial Mediastinitis, *Arch Int Med* **35** 362 (March) 1925

7 Groedel and Monckeberg. *Zentralbl f Herzkrankh u Gefasse* **5** 2 1913.

pulmonary artery This position throws the septum into the frontal plane, and places the left ventricle completely dorsal and the right ventricle completely ventral in relation to this plane Obviously, this condition was practically duplicated by our rotation of the heart around its longitudinal axis, without displacement around the anterior-posterior axis

The same authors have shown that a pendant heart may give the same electrocardiogram as a transverse heart, simply owing to this rotation around the longitudinal axis In cases of left sided hypertrophy due to mitral insufficiency, aortic lesions or sclerosis of the blood vessels of the kidney, the growth of the left ventricle is described as rotating the heart to the right around its longitudinal axis, thus making the left ventricle more ventral in position, and always producing a strongly positive initial deflection of the electrocardiogram Rotations of the ventricles around the longitudinal axis, whether due to experimental, physiologic, or pathologic causes, must obviously disturb the normal relationship of the muscle masses of both ventricles to the frontal plane The projection of the electrical vectors on this plane of the electrocardiographic leads is, of course, also greatly modified, and in this fact is the fundamental reason for the variation in the electrocardiographic curves

SUMMARY

1 Displacements of the heart around its anterior-posterior axis either to the right or the left as carried out experimentally do not give the curves anticipated according to the principle of the Einthoven triangle, or according to the accepted standards for right or left sided preponderance The electrical axis may be either increased or decreased or remain the same

2 If when the heart is displaced to the right or the left, a counter-rotation in the opposite direction around its longitudinal axis is brought about, the curves approach those of right and left sided preponderance, the electrical angle regularly increasing or decreasing as it should

3 It is evident that the effects of mere displacement have been masked by rotation on the longitudinal axis In proof of this it is found that, if the heart is held in its normal position and then rotated on its longitudinal axis, typical curves of right or left sided preponderance may be produced

4 Either uncomplicated rotation to the right on the anteroposterior axis or rotation to the left on the longitudinal axis will give curves characteristic of right sided preponderance The reverse conditions will give curves characteristic of left sided preponderance

5 The importance of having the heart in the normal position for all electrocardiograms is emphasized. It seems clear that it is impossible to tell how much a change in position may affect the electrocardiogram or how much an abnormal electrocardiogram may be due to variations in position, since the result is due to the summation of different rotations. Variations in position may accentuate, neutralize or reverse the effects of a slight degree of hypertrophy. It is for this reason that, as Heilmann and Wilson insist, the influence of the relative weight of the two ventricles predominates only when the heart is greatly hypertrophied.

VITAL CAPACITY, RESPIRATORY FREQUENCY, PULSE RATE AND SYSTOLIC BLOOD PRESSURE IN HEART DISEASE

THEIR IMPORTANCE IN THE CLASSIFICATION OF PATIENTS *

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A satisfactory classification of patients with organic heart disease based on their ability to carry on physical activity would be of great value to every clinician. It is our purpose in this article to show that the consideration of the vital capacity of patients with heart disease, together with their respiratory frequency, pulse rate and systolic blood pressure, after climbing 30 feet of stairs in forty seconds divides them into three main groups. The form of exercise test used in this study differs entirely from that type of test in which the individual is exercised until dyspnea first appears, in that a definite time and weight lifting task is accomplished. In this study the reaction of the individual to a definite task is noted instead of only the amount of work that may be done to bring about dyspnea in the individual. It is hardly necessary to state that the results obtained by these two methods are not comparable.

The Association of Cardiac Clinics of New York recognizes the following classification of patients with heart disease

Class I Organic—able to carry on habitual physical activity

Class II Organic—able to carry on

A Slightly diminished physical activity

B Greatly diminished physical activity

Class III Organic—unequal to any physical activity

Class IV Possible—having doubtful murmurs, mainly accidental, possibly organic

Class V Potential—having a predisposing history

Class I and Class II, A and B, are based on the ability of cardiac patients to carry on physical activity, however, up to the present time no uniform method has been described to aid us in grouping heart cases according to the foregoing classification. Various procedures are in use today. Some of these are based on the measurement of the vital capacity, others, on the pulse or systolic blood pressure reactions following different exercises, such as stair climbing, body bending, deep knee flexions and swinging of dumb-bells. The importance of completing

* From the Mineola Home for Cardiac Children, Irvington, N Y, in the service of Dr Robert H Halsey

uniform exercise has been recognized. Thus, there may be variations in the number of feet actually climbed or in the time allowed for climbing the same number of stairs, in the number of times the body bending exercises and the deep knee flexions are performed, and in the weight of the dumb-bells or in the time allowed for each swing. Obviously, comparable results cannot be expected since the actual effort required is not controlled by time or work to be accomplished.

Investigators have attempted to employ one or more of these tests as a means of determining the functional capacity of the heart, using either the estimation of the vital capacity or the time required for the pulse or systolic blood pressure to return to the rest levels.

The inferences drawn from these determinations are numerous and varied. Brittingham and White,¹ in studying the systolic blood pressure reaction after exercise, concluded that it may be of some value in determining the individual's tolerance for work. The results obtained, however, were not uniform in that cases with apparently no organic heart disease presented systolic pressure curves similar to those obtained in cardiac patients. Wilson² utilized the degree of distress and systolic pressure curves as a gage in estimating the exercise tolerance of children with heart disease, and found that exercise tolerance tests give important information which may be employed as a scientific basis for the proper regulation of the child's activities. In Propst's³ series of cases with organic heart disease only 3 per cent presented abnormal pressure reactions. He was more satisfied with the results obtained by following the changes in the pulse rate before and after exercise. Brady⁴ feels that a pulse not returning to the preexercise level in three minutes following fifteen deep knee flexions should be viewed with suspicion.

Similarly, various observers have stated that there was no uniform relation between the vital capacity of the lungs and the condition of the heart in patients with heart disease. The work of Peabody and Wentworth⁵ would indicate that there is a close relationship between the degree of dyspnea noted in heart disease and the vital capacity of the lungs. They found that in general patients with a vital capacity of 90 per cent or more of the normal standard have little tendency to dyspnea.

1 Brittingham, H. H., and White, P. D. Cardiac Functional Tests, *J. A. M. A.* **79** 1901 (Dec 2) 1922.

2 Wilson, May G. Exercise Tolerance of Children with Heart Disease, *J. A. M. A.* **76** 1629 (June 11) 1921.

3 Propst, D. W. Exercise Cardiac Functional Test in One Hundred Cases of Heart Disease, *J. A. M. A.* **82**:2102 (June 28) 1924.

4 Brady, William. The Anamnesis in Cardiovascular Disease, *J. A. M. A.* **70** 1449 (May 18) 1918.

5 Peabody, F. W., and Wentworth, J. A. Clinical Studies of the Respiration, IV, The Vital Capacity and Its Relation to Dyspnea, *Arch. Int. Med.* **20** 443 (Sept) 1917.

Patients with a vital capacity from 70 to 90 per cent of the normal become dyspneic following unusual exertion and must restrict their daily activities, but may perform light work. Patients with a vital capacity of from 40 to 70 per cent of the normal standard show marked dyspnea on slight exertion and are, as a rule, unable to work. Patients with a vital capacity of less than 40 per cent of the normal are decompensated patients. Brittingham and White¹ report a low vital capacity in 100 per cent of their patients with congestive heart failure, while, almost without exception, in those cases with no congestive failure a normal vital capacity was observed. Burton-Opitz⁶ emphasizes that the vital capacity changes little in compensated cases. Wilson and Edwards⁷ observed a reduction in the vital capacity in cardiac patients showing a diminished exercise tolerance. The group averages pointed to a relationship between the reduction in the vital capacity measurements and the degree of diminution in the exercise tolerance. Although this relationship has been observed in the group averages, no such definite relationship could be assigned to the individual cases.⁸

It is apparent that considerable difference of opinion exists with respect to the clinical value and to the proper interpretation of the results obtained when these tests are used as tests of the heart. Because of the marked variability noted, the majority of the tests have not won uniform approval and have been considered valueless by many clinicians. The majority of the investigators have made the error of using the tests as heart tests rather than as tests of the person with heart disease. It is obvious, from the experimental data cited and from our own observations, that not one of the tests can be considered a satisfactory or reliable heart test. However, when they are used collectively, with the object of testing the subject with heart disease, definite and valuable information is obtained.

Our study consisted of the estimation of the vital capacity at rest and the determination of the length of time required for the respiratory rate, pulse rate and systolic blood pressure to return to their preexercise level after the patients climbed 30 feet of stairs in forty seconds. All the patients tested were children between the ages of 6 and 16 years with definite organic heart disease due to the infection of rheumatic fever. The patients were ambulatory and showed none of the early symptoms of decompensation, such as precordial distress, edema of the ankles, or signs of pulmonary congestion. They presented a normal temperature and, as far as could be ascertained, were free from any

6 Burton-Opitz, Russell. Vital Capacity of "Cardiacs," *J A M A* **78** 1686 (June 3) 1922

7 Wilson, May G., and Edwards, D. J. The Vital Capacity of the Lungs and Its Relation to Exercise Tolerance in Children with Heart Disease, *Am J Dis Child* **22** 443 (Nov.) 1921

8 Wilson and Edwards (Footnote 7) Ziskin, Thomas. Vital Capacity as a Functional Test in Heart Disease, *Arch Int Med* **35** 259 (Feb.) 1925

other disease. The respiratory rate and the pulse rate were taken immediately after the exercise and at one minute intervals until the respiratory rate was within two points and the pulse rate within four points of the preexercise level. The systolic blood pressure was taken immediately after the exercise and at one-half minute intervals until it reached within 6 mm. of the pressure at rest. A series of ninety-six cases was studied. The results obtained are recorded in the table.

An examination of the table shows that the cases studied may readily be classified into three main groups. The patients in the first group show a vital capacity of not less than 90 per cent. of the normal and a return of the respiratory rate, pulse rate and systolic blood pressure to the rest level within two minutes' time. The patients in the second group show either a vital capacity of from 90 to 80 per cent. of the normal standard or an increase in the time required for any one of the factors concerned in the exercise tests to return to the preexercise level. This level was reached within three minutes' time. The patients in the third group show either a vital capacity of less than 80 per cent. of the normal or a period of time greater than three minutes required for either the respiratory rate, pulse rate, or systolic blood pressure to return to the preexercise level.

The patients coming under the first group show a vital capacity within normal limits and a return to the rest level in two minutes of all the factors studied in the exercise test. These children were able to carry on usual physical activity without showing signs of dyspnea or overactivity of the heart and accordingly did not need to be restricted in their daily routine. Cases classified in this way correspond to Class I cases of the classification of the Association of Cardiac Clinics. The patients in the second group show either a vital capacity between 90 and 80 per cent. of the normal or a period of time greater than two minutes, but not more than three minutes for either one of the other factors to return to the preexercise level. The members of this group usually presented varying degrees of dyspnea and exhaustion, or some overactivity of the heart following exertion caused by setting-up exercises, stairs climbing, etc. They may conveniently be placed in Class II A of the association classification. The children coming under the third group show either a vital capacity below 80 per cent. of the normal or require more than three minutes for any one of the other three factors to reach the rest level. The subjects in this group definitely presented signs of dyspnea, exhaustion, or overactivity of the heart after climbing 30 feet of stairs. These cases answer very favorably the description of the Class II B cases of the association classification. In our series eight belong to the first group, forty to the second and forty-eight to the third group.

Vital Capacity and Respiratory Frequency, Pulse Rate and Systolic Blood Pressure Before and After Climbing Thirty Feet of Stairs in Forty Seconds

Case	Diagnosis	Class	Vital Capacity Percentage of Normal	Exercise Test																	
				Respiratory Rate					Pulse Rate					Systolic Blood Pressure							
				At Rest	Minutes After Exercise				At Rest	Minutes After Exercise				At Rest	Minutes After Exercise						
					1	2	3	4		5	1	2	3		4	5	1	2	3	4	5
1	Mitral insufficiency	I	105	24	32	24				86	104	88				110	118	108			
2	Mitral insufficiency and stenosis	I	102	20	28	24				92	100	70				114	142	130	120		
3	Aortic insufficiency and mitral insufficiency	I	93	20	28	22				96	120	100				110	150	132	114		
4	Mitral insufficiency	I	103	24	28	24				84	108	86				118	158	132	124		
5	Mitral insufficiency	I	96	20	28	24				88	104	88				130	158	136	132		
6	Aortic insufficiency	I	127	24	32	28				100	150	98				150	180	160	150		
7	Mitral insufficiency and stenosis	I	98	20	28	24				98	124	102				106	132	118	110		
8	Mitral insufficiency and aortic insufficiency	I	92	24	28	24				84	120	76				134	162	148	140		
9	Mitral insufficiency	II A	88	24	28	24				84	100	80				122	180	150	130	122	
10	Mitral insufficiency	II A	82	18	26	18				84	98	78				102	114	110	104		
11	Mitral insufficiency	II A	80	18	30	22	20			82	82					90	108	106	98	94	
12	Aortic insufficiency	II A	80	24	26	28	24			94	138	92				132	190	160	136		
13	Mitral insufficiency and stenosis	II A	82	24	28	24				118	136	122	120			100	122	116	108	100	
14	Aortic insufficiency and mitral insufficiency	II A	80	28	36	32	28			80	106	74				110	200	150	122	110	
15	Aortic insufficiency, mitral insufficiency and stenosis	II A	85	24	28	26				84	102	82				110	136	120	116	112	
16	Mitral insufficiency	II A	82	20	28	21	20			94	116	96				132	158	140	134		
17	Aortic insufficiency and stenosis, mitral insufficiency and stenosis	II A	90	24	36	32	28			78	100	76				126	138	124			
18	Aortic insufficiency, mitral insufficiency and stenosis	II A	88	24	28	24				82	98	78				124	150	134	130		
19	Aortic insufficiency and stenosis, mitral insufficiency and stenosis	II A	81	24	32	28	24			92	126	100	94			130	164	160	148	136	
20	Aortic insufficiency, mitral insufficiency and stenosis	II A	89	21	28	24				86	101	84				98	168	140	118	104	
21	Aortic insufficiency, mitral insufficiency and stenosis	II A	80	20	28	24	22			88	104	84				122	158	138	124		
22	Mitral insufficiency	II A	80	20	32	28	22			82	94	80				96	118	120	100		
23	Aortic insufficiency and stenosis, mitral insufficiency and stenosis	II A	81	20	28	24	22			90	118	108	92			140	162	156	140		
24	Aortic insufficiency, mitral insufficiency and stenosis	II A	89	20	28	24	20			104	140	106				116	134	130	116		
25	Mitral insufficiency	II A	85	18	28	24	20			78	98	90	80			116	144	130	114		
26	Mitral insufficiency and stenosis	II A	90	24	36	32	26			82	102	80				136	160	148	134		
27	Mitral insufficiency and mitral insufficiency	II A	88	20	30	28	24			82	104	82				92	102	100	94		
28	Aortic insufficiency and mitral insufficiency	II A	84	20	28	24				70	104	72				106	138	118	110		
29	Mitral insufficiency	II A	82	22	26	24				88	130	90				110	124	120	116	112	
30	Aortic insufficiency and mitral insufficiency	II A	85	21	32	28	24			82	106	78				140	200	180	150	142	
31	Mitral insufficiency and stenosis	II A	88	24	28	24				84	120	78				112	136	130	118	112	
32	Aortic insufficiency and stenosis, mitral stenosis	II A	88	24	28	24				92	130	98	90			131	162	150	140	134	
33	Aortic insufficiency, mitral insufficiency and stenosis	II A	91	24	28	24				90	102	90				120	150	138	128	122	
34	Mitral insufficiency and stenosis	II A	85	21	28	24	22			84	108	90	86			92	102	92			
35	Mitral insufficiency	II A	89	20	32	26	22			82	112	84				98	144	120	104		
36	Mitral insufficiency	II A	80	16	22	22	18			88	100	84				98	130	116	98		
37	Mitral stenosis	II A	87	24	26	24				88	100	84				128	148	140	134		
38	Mitral insufficiency and stenosis	II A	80	20	26	28	24			90	140	116	88			118	152	146	130	122	
39	Aortic insufficiency	II A	82	24	28	24				88	128	86				138	188	148	140		
40	Aortic insufficiency	II A	80	24	36	28				88	128	78				140	182	172	158	144	
41	Aortic insufficiency	II A	87	24	32	24				92	138	110	94			116	138	120			
42	Mitral insufficiency	II A	82	24	28	24				86	108	88				111	140	118	118		
43	Mitral insufficiency	II A	80	20	24	20				96	120	108	92								

43	Mitral insufficiency	II A	88	18	28	21	20	96	140	90	108	134	120	110
44	Aortic insufficiency	II A	80	20	28	21	20	88	112	88	126	142	122	
45	Aortic insufficiency and stenosis	II A	87	24	28	23	21	88	120	90	122	162	138	122
46	Mitral insufficiency	II A	90	21	32	20	20	92	112	88	110	138	130	120
47	Aortic insufficiency, mitral insufficiency and stenosis	II A	87	21	44	26	20	70	98	88	120	138	134	124
48	Aortic insufficiency and mitral insufficiency	II A	80	23	36	32	28	94	114	108	104	130	116	112
49	Mitral insufficiency and stenosis	II B	77	42	10			60	120	54	92	134	130	110
50	Mitral insufficiency	II B	73	28	48	46	40	96	156	120	116	104	100	98
51	Mitral insufficiency	II B	65	21	32	20	20	96	132	106	106	132	128	122
52	Aortic insufficiency, mitral insufficiency and stenosis	II B	72	20	30	23	24	106	122	104	102	132	122	112
53	Mitral insufficiency	II B	88	16	24	22	20	90	104	88	110	154	138	124
54	Mitral insufficiency and stenosis	II B	62	16	20	16		78	104	88	84	110	108	94
55	Mitral insufficiency	II B	72	16	24	22	18	100	132	120	116	110	96	110
56	Mitral insufficiency	II B	70	18	24	20	24	68	90	60	114	116	140	122
57	Mitral insufficiency	II B	78	18	28	24	24	84	128	102	110	146	200	180
58	Aortic insufficiency, mitral insufficiency and stenosis	II B	52	24	22	28	26	100	126	122	144	200	200	189
59	Aortic stenosis, mitral insufficiency and stenosis	II B	76	18	24	20		112	128	120	100	110	118	106
60	Mitral insufficiency and stenosis	II B	30	28	36	30	28	88	140	120	88	152	118	100
61	Mitral insufficiency and stenosis	II B	62	28	30	32	32	112	130	118	102	114	102	94
62	Mitral insufficiency	II B	70	20	28	24	20	88	120	82	118	172	132	128
63	Mitral insufficiency	II B	65	24	32	28	24	104	116	102	110	136	118	112
64	Aortic insufficiency, mitral insufficiency and stenosis	II B	64	24	28	24		96	130	100	80	100	96	78
65	Aortic insufficiency, mitral insufficiency and stenosis	II B	63	22	32	28	24	92	170	140	114	132	142	140
66	Mitral insufficiency and stenosis	II B	45	30	52	48	30	108	130	116	102	128	110	104
67	Aortic insufficiency and mitral insufficiency	II B	60	21	32	28	24	108	130	110	98	122	116	112
68	Aortic insufficiency and mitral insufficiency	II B	50	24	32	28	28	96	142	108	106	126	130	120
69	Mitral insufficiency	II B	70	20	24	24	20	88	100	84	122	170	158	148
70	Aortic insufficiency and mitral insufficiency	II B	85	20	28	24	20	74	96	76	134	196	174	156
71	Aortic insufficiency and mitral insufficiency	II B	70	24	32	28	24	68	140	90	92	112	92	132
72	Aortic insufficiency, mitral insufficiency and stenosis	II B	45	24	32	28	28	94	116	102	132	162	144	128
73	Aortic insufficiency, mitral insufficiency and stenosis	II B	58	24	40	36	28	108	140	104	132	154	154	140
74	Aortic insufficiency, mitral insufficiency and stenosis	II B	70	24	32	28	24	92	140	118	112	132	144	130
75	Mitral insufficiency	II B	50	24	38	32	28	86	108	88	112	128	114	122
76	Aortic insufficiency and mitral insufficiency	II B	50	24	44	40	36	118	160	138	124	126	134	122
77	Aortic insufficiency and mitral insufficiency	II B	75	20	28	24	24	92	140	122	120	138	130	128
78	Aortic insufficiency and mitral insufficiency	II B	70	20	22			88	112	86	124	140	136	126
79	Aortic insufficiency and mitral insufficiency	II B	73	20	30	24	20	80	108	84	110	138	126	112
80	Mitral insufficiency and stenosis	II B	62	24	44	36	32	102	128	124	112	130	124	122
81	Mitral insufficiency	II B	71	22	26	24	20	78	144	100	122	140	124	120
82	Aortic insufficiency and mitral insufficiency	II B	55	20	28	22		110	130	120	122	142	132	120
83	Aortic insufficiency and mitral insufficiency	II B	85	22	28	24	24	108	140	120	160	200	198	186
84	Mitral insufficiency	II B	63	24	28	28	24	88	110	92	108	120	110	170
85	Aortic insufficiency and mitral insufficiency	II B	45	24	32	28	28	104	154	122	110	160	160	152
86	Aortic insufficiency and mitral insufficiency	II B	30	24	30	24	28	96	112	100	114	144	124	142
87	Mitral insufficiency and stenosis	II B	62	20	30	24	22	92	124	120	116	134	120	118
88	Aortic insufficiency, mitral insufficiency and stenosis	II B	45	28	32	30	28	96	124	108	102	150	138	126
89	Mitral insufficiency and stenosis	II B	72	20	30	24	20	68	88	66	100	126	110	100
90	Mitral insufficiency	II B	50	40	46	28	20	120	150	140	124	152	132	124
91	Mitral insufficiency and mitral insufficiency	II B	62	24	28	26	24	120	170	140	114	134	126	120
92	Aortic insufficiency and mitral insufficiency	II B	70	24	36	32	28	90	112	104	114	132	128	120
93	Aortic insufficiency and mitral insufficiency	II B	62	24	28	24	28	80	114	80	110	130	120	112
94	Mitral insufficiency	II B	60	24	28	24		96	122	98	98	120	94	
95	Mitral insufficiency	II B	52	24	28	24	24	84	86		106	130	110	
96	Aortic insufficiency, mitral insufficiency and stenosis	II B	72	18	24	20	20	104	140	120	114	144	138	128

When we undertook our study we were aware of the lack of a uniform method of classifying patients with organic heart disease. We were likewise aware of the criticisms directed at the various tests employed as cardiac function tests or exercise tolerance tests. In our work we used practically the same tests utilized by other observers, however, we attached a different significance to them. The majority of the investigators employed either the measurement of the vital capacity or the pulse and systolic pressure curves as tests of the heart. The results were generally disappointing, and for that reason the tests were considered as being of little or no value by many clinicians. It is quite obvious that these tests are not heart tests. It is well known that the vital capacity, respiratory frequency, pulse rate and systolic pressure are influenced by fright, excitement, neurotic tendencies and in some degree by the physical condition of the patient. For that reason, many non-cardiac patients may present the same reactions observed in heart cases. This fact, however, does not prove that the tests are absolutely valueless in the study of patients with heart disease. When used for the purpose of testing the individual with cardiac disease, rather than of testing the heart of the patient, helpful information is obtained. The tests must be regarded then as tests of a subject and not as tests of the subject's heart. On the basis of the vital capacity and the response of the patient to exercise as measured by the respiratory frequency, pulse rate and systolic pressure, these cases group themselves into the three main classes described above regardless of the valvular damage.

A close analysis of the results presents another important point, the lack of a general parallelism between the various factors studied. It will be noted that many cases presenting a prolonged fall in the pulse rate may have a normal blood pressure curve and vice versa. Other cases showing a prolonged fall in either the pulse rate or systolic pressure have a vital capacity within normal limits, or a low vital capacity may be obtained with a normal pulse and systolic pressure curve. That would indicate that the estimation of the vital capacity alone, or the determination of either the respiratory frequency, pulse rate or systolic pressure alone is not a reliable test of the subject but that complete information concerning the case can only be obtained by a utilization of all the observations.

The method of classification we have adopted can be defined clearly and is not influenced by the personal equation of the persons performing the tests. It affords a more uniform way of classifying patients with heart disease than has previously been described. Such a classification enables the physician to regulate and prescribe more intelligently the physical activity of the patient with heart disease.

SUMMARY AND CONCLUSIONS

On the basis of the measurement of the vital capacity of ninety-six children with organic heart disease, together with their response to the climbing of 30 feet of stairs in forty seconds as measured by the respiratory frequency, pulse rate and systolic blood pressure, these cases can be conveniently grouped in three main classes corresponding to Class I, Class II A and Class II B of the classification of the Association of Cardiac Clinics. Because of the lack of a general parallelism between the results of the various tests, it is evident that one test alone does not furnish a satisfactory basis for making a classification of patients with heart disease. These tests cannot be considered cardiac function tests but are of great assistance in the intelligent care of children with organic heart disease.

CLINICAL DIAGNOSIS AND THE INTESTINAL FLORA *

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For clinical purposes, the determination of the intestinal flora should be made in the shortest possible time. The work takes forty-eight hours which is the shortest average time for giving the findings required for such an examination. This is not intended for a detailed bacteriologic study on all bacteria present in the flora, but consideration is centered on a few bacterial groups whose dominance is directly responsible for the character of the flora. The elaborate study of intestinal bacteriology advanced by McNeal,¹ Meyer and others² is rather cumbersome for clinical diagnosis, while the method of Rettger and Cheplin,³ although simple, does not cover the entire field. In developing the method, I have carefully scrutinized cultural and morphologic characteristics of the dominant bacteria in question, thereby making it possible to draw accurate interpretation of the floral findings.

The bacteria in the stool are numerous and constitute from one fourth to one third of fecal dry matter. There are at least 100 different kinds of bacteria present. The morphologic and cultural characteristics of some of these are as yet unknown to us. The isolation of each of these bacteria requires much time, and from the clinical standpoint is not worth it. My aim is, therefore, not to determine one bacterium or two, but to diagnose the intestinal flora as a whole whether it be of fermentative, putrefactive or mixed type.

The bacteriologic examination of the stool consists of cultural and microscopic technic, and the character of the flora is determined by the findings, the emphasis being laid on the cultures. In other words, the diagnosis is made from the summarized results by checking one against the other. In many cases the microscopic examination helps to substantiate the cultural findings.

In this study the intestinal bacteria are classified under the following groups

- (a) Colon-like bacteria (*Bacillus coli*, *B. proteus-vulgaris*, *B. aerogenes*, *B. enteritidis*, *B. fecalis-alkaligenes*, etc.),
- (b) Acidophilus-like bacteria (*B. acidophilus*, *B. bifidus*),

* From the clinical laboratories, Battle Creek Sanitarium

1 McNeal et al. J Infect Dis 6 103, 571, 1909

2 Morris, G. B., Porter, R. L., and Meyer, K. A. J Infect Dis 25 349 (Nov) 1919

3 Rettger and Cheplin. A Treatise on the Transformation of the Intestinal Flora, New Haven, Conn., Yale University Press, 1921

(c) Spore-bearing bacteria (*B welchii*, *B putrificus*, *B butyricus*, etc),

(d) Gram-positive cocci (*Streptococcus fecalis*, micrococci, diplococci and enterococci), and

(e) Yeasts

For diagnostic purpose the intestinal flora is classified under the following headings

(a) Fermentative This type contains a large percentage of *Bacillus acidophilus*, and is excellent in every respect Fermentation does not imply an excessive gas production The percentage of coli-like organisms is very small

(b) Semifermentative This type contains a fairly large number of *Bacillus acidophilus* with some coli-like bacteria with or without streptococci

(c) Mixed This type is a congregation of all sorts of bacteria A small percentage of *Bacillus acidophilus* and *Bacillus welchii* are often present in this type

(d) Semiputrefactive This type shows the dominance of putrefactive bacteria with or without *Bacillus acidophilus*

(e) Putrefactive This type is characterized by being distinctly putrefactive, dominated by the *Bacillus coli* group and spore-bearing without the presence of *Bacillus acidophilus* The bacterial metabolism is strongly proteolytic

TECHNIC OF BACTERIOLOGIC ANALYSIS

Fecal Suspension—This is prepared by thoroughly mixing about a pea-sized stool or 1 c c, if liquid, with 10 c c of sterilized physiologic sodium chlorid solution to make a uniformly homogeneous emulsion A small ball of cotton should be inserted clear to the bottom of the tube to serve as an automatic filter The supernatant suspension thus made is devoid of food remnants, which often interfere with technic

Microscopic Examination—(a) Smear The uniform smear is made from the suspension by means of a platinum wire, and dried either by flame or air The slide is stained according to the Gram method

(b) Gram Stain Classification of Bacteria This is made by examining at least from ten to fifteen microscopic fields with the aid of a square field ocular, and calculating the percentage of gram-positive and negative Special attention is paid to the presence of acidophilus-like organisms that are of polymorphous rod shape If there is dominance of a particular type of bacteria, note is made by the plus sign For the convenience of the study, the following bacterial groups are placed in the working blank coli-like bacteria, acidophilus-like bacteria, spore-

bearing bacteria, streptococci and yeasts Under these headings the following bacteria are gram-positive *B acidophilus*, *B welchii*, *B butyricus*, *B putrificus*, streptococci and diplococci Most other bacteria are gram-negative

Microscopic Morphology of *B Acidophilus* This should be carefully studied, as the determination of the flora depends entirely on the findings of this particular organism It is a gram-positive rod and polymorphous They generally appear singly, but often in pairs or in a short chain The single bacillus is a slender rod, often slightly tapering at both ends and resembling a part of the corkscrew-like formation of spirocheta, and again it may present almost a coccus-like appearance The smear made from an old culture often fails to retain gram iodine, and gives an appearance of a gram-negative organism *B acidophilus* does not possess spore, capsule or flagella They are immotile

Morphology of Colon-Like Bacteria All the gram-negative bacteria of every description are included in this category

Morphology of Spore-Bearing Type They are short and thick rods, usually found in pairs but often singly The consideration of *butyricus* and *putrificus* should be more or less dependent on the cultural findings

The higher the percentage of gram-positive bacteria, as a rule, the better the condition of the intestinal flora, while with the higher percentage of gram-negative, the worse the condition, provided the cultural findings coincide with the microscopic A higher percentage of gram-positive, therefore, does not necessarily indicate, in all cases, fermentative flora, as there are often cases in which the microscopic fields are crowded with gram-positive bacteria of the spore-bearing type and diplococci However, if there is a predominance of gram-positive bacteria coupled with good cultural findings, it is safe to make a favorable diagnosis of the flora The microscopic test is not, therefore, an accurate method, and should be wholly or partially dependent on the cultural findings The higher percentage of gram-negative bacteria is always found in putrefactive flora The approximate percentage of gram-positive bacteria in different types of intestinal flora is Fermentative, from 75 to 100, semifermentative, from 55 to 70, mixed, from 35 to 50, semiputrefactive, from 10 to 30, and putrefactive, from 5 to 20

Cultural Examination—**Litmus Milk Culture** The test tube used for this purpose is 1 inch (2.5 cm) in diameter and 6 inches (15.2 cm) in length A small inverted tube is inserted in order to determine the percentage of gas formation The media is distributed in culture tubes, and sterilized in the autoclave at 15 pounds pressure for 20 minutes, 1 c.c. of fecal suspension is inoculated into milk culture, and incubated at 37 degrees C. for 48 hours

Reaction The reduction of litmus by the acidity formed by bacteria is designated by plus signs according to the degree of acidity. Whether the flora is putrefactive or fermentative, the reaction is always acid except in the cases of special types of bacteria, such as *B. typhosus*, *B. dysenteriae* and *B. fecalis-alkaligenes*. The longer the incubation, the higher the production of acidity. *B. coli* and *B. acidophilus* as well as *B. welchii* intensify the acidity in prolonged incubation.

Coagulation This is classified under the following headings:

(a) Cheeselike or stormy fermentation. The coagulated milk presents lattice-like furrows. If accompanied by floating gas bubbles with a large amount of whey, the presence of *B. welchii* is suspected. This is confirmed by a special test described later.

(b) Soft curd. Yellowish soft curd with or without whey formation is indicative of putrefactive flora dominated by *B. proteus vulgaris*. If such a culture is left incubated for a longer period, a complete peptonization takes place.

(c) Retraction. Complete retraction of the curd is a putrefactive process. Partial retraction is often found in any type of flora. The interpretation varies according to other findings in milk.

(d) Firm massive coagulation. The firm massive coagulation with whey indicates the fermentative type.

If the flora is distinctly dominated by *B. acidophilus*, the coagulation is slimy at twenty-four hours' incubation. On further incubation, the acidity of the milk increases owing to the formation of lactic acid from lactose, and consequently there is a production of whey. The amount increases as the incubation is prolonged.

Whey The amount of whey is designated by a plus sign. The test tube is tilted slightly to measure the approximate amount.

The interpretation of whey varies according to other findings. If accompanied by stormy fermentation it indicates the presence of spore-bearing bacteria, notably *B. welchii*, with firm massive coagulation, the presence of the fermentative type is indicated. The complete peptonization is a putrefactive process.

Gas Formation The inner tube takes the place of an ordinary fermentation tube used elsewhere. It is less expensive, and for routine procedure it answers the purpose just as well. The whole length of the tube is considered as 100 per cent. If one half the tube is filled with gas, it is considered as 50 per cent; one fourth, 25 per cent.

The amount of gas in the fermentative type is probably low, ranging from 0 to 20 per cent, while in the putrefactive flora it is higher, often almost 100 per cent.

Summarizing the findings obtained from milk culture in the typical cases, putrefactive flora is characterized either by stormy fermentation

Interpretation of Intestinal Flora

Type	Putrefactive	Semiputrefactive	Mixed	Semifermentative	Fermentative
Infant's milk	Stormy fermentation or soft curd, floating gas bubbles, complete peptonization, large percentage of gas in inner tube, slight reduction of litmus	Soft curd with or without whey, moderate percentage of gas, putrid reaction, accompanied by stormy fermentation	Soft curd or firm coagulation, with or without whey, moderate percentage of gas, fair reduction of litmus	Firm massive coagulation with moderate amount of whey, small percentage of gas, reaction acid	Firm massive coagulation with whey, 0-10 per cent of gas in inner tube, reaction distinctly acid
Confluent	B welchii positive	B welchii positive	B welchii positive or negative	B welchii negative	B welchii negative
Lactose broth	Turbidity, with scum and pellicle or sediment, gas in inner tube, 40-60 per cent	Turbidity, with scum, pellicle or sediment, gas in inner tube, 40-60 per cent	Turbidity, with or without scum pellicle sediment moderate, gas in inner tube, 25-40 per cent	Turbidity with or without scum or pellicle, gas 5-25 per cent according to the number of B coli	Turbidity slight, with or without scum or pellicle, gas 0-10 per cent according to the number of B coli
Veillon tube	Large percentage of gas consisting of colon and welchii types, with or without acidophilus colony	Large percentage of gas consisting of colon and welchii types, with or without acidophilus colony	Fair amount of gas dominated by coli type, some acidophilus colonies	No gas or little of B coli group, moderate number of acidophilus colonies	No gas formation. Abundant acidophilus colonies
Petri plate	No acidophilus colony present, other colonies numerous and variable	With or without acidophilus colony, other colonies are quite numerous and variable	Some acidophilus colonies, other colonies fairly variable	Many acidophilus colonies, few other types	Abundant acidophilus colonies a very few other types
Microscopic examination	Gram positive, 0-30 per cent predominance of coli group with spore bearers and occasional gram negative abundant	Gram positive, 10-30 per cent, a few rod shaped acidophilus like bacilli, spores often scattered, B welchii present	Gram positive 35-50 per cent, acidophilus like bacilli mixed with coli type, B welchii in small number or absent	Gram positive, 35-70 per cent, presence of many acidophilus like bacteria, total absence of spore bearers	Gram positive, 75 per cent up, presence of abundant acidophilus like bacteria with a few coli group, no spore bearers
Microscopic examination	Serbylla or formed liquid or semiliquid putrid hydro-gen sulphid ammoniac or butyric odor, dark brown mucus often present, with or without blood	Formed or liquid putrid odor, dark brown, mucus often present, with or without blood	Partly formed or mushy, no odor or slightly putrid, color variable, with or without mucus or blood	Putty formed or mushy, no odor or sour, green or yellow, no mucus, no blood	Fluffy, mushy or partly formed, sour or no odor, green or yellow, no mucus, no blood
Chemical tests	Reaction alkaline, acid or neutral	Reaction alkaline, acid or neutral	Occult blood seldom present	Occult blood negative	Occult blood negative
Parasites	Present or absent	Present or absent	Absent	Absent	Absent

or yellow soft curd with considerable whey and gas bubbles, accompanied by 40 per cent or more of gas in the inner tube. Fermentative flora shows a firm massive coagulation with some whey and a small percentage of gas or none at all in the inner tube with a distinctly acid reaction. An analysis of the types of flora is given in the accompanying table. Emphasis is therefore placed chiefly on the types of coagulation and of the peptonizing process.

One Per Cent Lactose Broth. The reaction is adjusted to 0.7 per cent of acidity according to an ordinary titration method, and the mediums are distributed in test tubes. The small tubes are inserted as in the milk culture for a similar purpose. It is autoclaved at 15 pounds pressure for twenty minutes. One-half cubic centimeter of fecal suspension is inoculated into each broth culture and is incubated at 37 degrees C. for forty-eight hours.

Turbidity. This is designated by plus signs according to its degree. The presence of the coli group, streptococci and enterococci induces an intense turbidity, while the flora dominated by acidophilus shows a smaller degree of turbidity.

Formation of Scum, Sediment and Pellicle. In the putrefactive type, these formations are noticeable especially if dominated by the coli group and spore-bearing bacteria. Sediment is observed when the streptococci group plays a dominant part. In the fermentative type, their formation as a rule is scarce, except in the case of a luxuriant growth of yeasts when a thick pellicle frequently develops.

Gas Formation. The percentage of gas is computed as in the case of a milk culture. In the putrefactive type, the percentage always increases and often reaches as high as 90 per cent. If there is over 60 per cent of gas in the broth culture coupled with the characteristic appearance of a stormy fermentation in the milk culture, it is safe to determine the dominance of *B. welchii*. In the fermentative type, in which *B. acidophilus* plays an extensive part, the gas formation is reduced to a minimum. The hydrogen ion concentration of the mediums is brought to such an extent that the growth of the gas forming bacteria is partially or totally suppressed owing to the excessive evolution of acetic and lactic acid by aciduric bacteria.

Summarizing the findings obtained from the broth culture, we may say that the putrefactive type is characterized by the presence of intense turbidity with the formation of a scum and pellicle accompanied by a large percentage of gas in the inner tube. Over 60 per cent, sometimes indicates the presence of *B. welchii*. The fermentative type shows turbidity to a small extent with or without scum or pellicle and accompanied by a small percentage of gas.

Veillon Culture. A tubing 12 inches (30.4 cm.) in length is used for the culture. One end of the tube is tightly plugged with an unper-

forated rubber stopper, and the other end with a ball of cotton. The medium used is 1 per cent lactose agar. The reaction is adjusted to 0.7 per cent of acidity. The tube is filled with medium 9 inches (22.8 cm) in depth and is sterilized in the autoclave at 15 pounds pressure for twenty minutes. One loopful of suspension is inoculated after the medium is cooled down to 42 degrees C, and the tube is tilted back and forth rather quickly in order that the inoculum will diffuse through the entire tube. The tube is dipped into cold water after the inoculation and is incubated at 37 degrees C for from twenty-four to forty-eight hours. When the tube is placed in the incubator, a horizontal position is preferable for the culture to maintain uniform distribution of growth of gas forming and non-gas forming bacteria.

The Veillon culture was originated by Veillon⁴ to determine the anaerobic growth, but for this purpose the tube answers three conditions. It favors (1) anaerobic bacterial growth, especially stormy fermentation of *B. welchii*, (2) the gas formation of facultative anaerobic bacteria, namely, those of colon-like organism, and (3) non-gas formation of *B. acidophilus*. If the deeper part of the medium is filled with big gaps, the presence of *B. welchii* can be suspected. In many instances, the gas evolved by the fermentative activity of *B. welchii* is so vigorous as to push the plug clear up to the top and break the medium asunder, as shown in Figure 2. The small bubbles of gas present throughout the culture are of *B. coli* and *B. aerogenes*. If there is no gas formation at all and the culture is occupied by many small fuzzy colonies, mostly microscopic, and other colonies of different shapes, it is safe to conclude that there is a decided predominance of non-gas forming bacteria. Some of these colonies are those of *B. acidophilus*.

Summarizing the findings obtained from Veillon culture, we may say that in the putrefactive type of flora there is a large amount of gas, especially of anaerobic type, in the mixed type there is a large amount of gas chiefly of colon-like bacteria with acidophilus colonies scattered throughout to a certain extent, and in fermentative type, there is no gas formation, but many minute fuzzy colonies of *B. acidophilus*.

In order to ascertain the identity of *B. acidophilus* colonies, the plate is made from the same medium as the foregoing or preferably 2 per cent dextrose agar. This will enable the examiner to eliminate the colonies of other non-gas forming organisms.

Lactose or Dextrose Agar Plate. A fecal suspension, 0.5 c.c., is inoculated into the medium, and is poured into the plate. The streak method is not desirable for this particular examination. Two aerobic plates are prepared for each specimen, and incubated for forty-eight hours at 37 degrees C.

⁴ Veillon. Arch de med exper et d'anat Path 10 517, 1898

The plate is made primarily for the determination of colonies of *B. acidophilus*. The colonies of *B. acidophilus* are different for each individual, and at different times in the same individual. There are no corresponding colonies in all the specimens examined. Thus, briefly, so far as my experience goes with this particular organism in regard to its characteristic colony formation, there are at least the following varieties:

Fimbriated type It is characterized by a microscopic fuzzy colony of unusually compact form, resembling bits of wool interlaced with radiating threads. The colony resembles that of *B. tetanus*. Under this group, there are several subdivisions:

(a) Irregular shaped nucleus, dense or comparatively transparent with numerous fimbriations.

(b) Oval or round colonies with few or numerous fimbriations. The nuclear matter is somewhat granular.

(c) A very small colony, usually irregularly shaped, and at times slightly triangular with a few fimbriations. When this species is being examined, care should be taken not to confuse it with the artefact present in the plate.

(d) Conglomerate type with chains of two, three or several colonies, each having a sort of bottle shaped structure with a few fimbriations. The conglomeration is mostly longitudinal, but seldom transverse.

Nonfimbriated type It is characterized by an oval or round colony without fimbriation. It is also microscopic, and if we carefully focus very short fimbriation may sometimes be detected, but not always. Whether or not these two main types of colonies are separate entities has not been fully brought out. I incline, however, to the belief that they are the same except under different concentrations of agar. Thus, if the agar is concentrated higher than 2.5 per cent, the pressure is brought to bear on the colonies to such an extent that the characteristic fimbriation may not be recognized. The hydrogen ion concentration of the medium as well as that of the fecal suspension is probably another important factor to be considered in this connection.

The percentage of *B. acidophilus* colonies is taken in proportion to the number of other colonies present in the plate. For accurate work, different dilutions should be prepared to facilitate the work. As the dilution increases, the number of colonies present becomes less, and the computation of the percentage of the acidophilus colonies can be made very easily. For instance, if there are 100 mixed colonies present, and ten are those of *B. acidophilus*, the percentage of the latter is 10. It is necessary to examine at least ten different microscopic fields on the plate, and make the average count of percentages. For routine purposes, the percentage of *B. acidophilus* colonies is conveniently designated by few, many and abundant.

The study is also made of the relationship of the various kinds of colonies with the type of the intestinal flora. Thus, in the putrefactive type, there is wide variation—a congregation of different types of bacteria, while in the fermentative type, the variety is reduced. In other words, the reduction of the variety of colonies present means a step by which the improvement of the flora takes place.

Summarizing the findings obtained from the plate, we may say that in the putrefactive type there is no acidophilus colony present and the variety of colonies is numerous, while in the fermentative type there are numerous acidophilus colonies with a few other kinds of colonies on the plate.

Confirmatory Test for *B. Welchii* Litmus milk is used for the culture. About 5 c c of the medium is distributed into each tube, and a small amount of liquid petrolatum is added in order to make the culture favorable for anaerobic growth. Then it is sterilized at 15 pounds pressure for twenty minutes. Fecal suspension, 0.5 c c, is inoculated

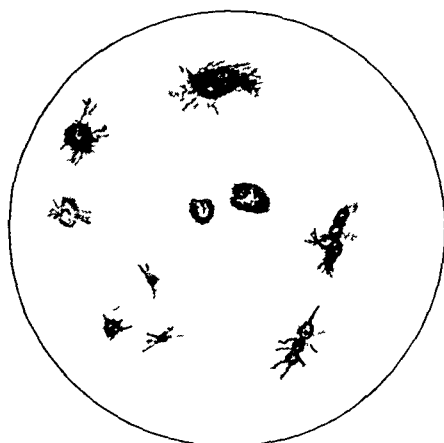


Fig. 1—Various colonies of *Bacillus acidophilus* (sketch)

into the tube, and is heated in a water bath at 80 C for twenty minutes to kill off vegetative forms of flora. This is incubated at 37 C for two days.

If *B. welchii* is present, characteristic stormy fermentation takes place by the breaking up of the coagulated casein accompanied by the butyric odor. The evolution of gas bubbles can be detected by shaking the tube gently.

The findings in milk, broth and Veillon cultures can be confirmed well by employing the foregoing method. In case there is a slight doubt as to the character of the gas formation in the Veillon tube, this test will help to differentiate *B. welchii* from the gas formation caused by *B. aerogenes* or its allied bacteria.

Although it is generally admitted that *B. welchii* is a strictly fermentative bacteria of saccharolytic type, as advanced by Rettger,⁵ its

5 Rettger J Biol Chem 11 71, 1906

presence in the cultural and microscopic examination is always indicative of putrefactive flora. In all the decisively fermentative type of flora there is hardly any *B. welchii* present. In the combination of coli group bacteria with *B. welchii*, the putrefactive process is very much augmented. According to Herter,⁶ *B. welchii* is one of the most important factors of intestinal putrefaction, being responsible for the so-called saccharobutylic fermentation. As the flora gradually improves, the percentage of *B. welchii* and other spore-bearing bacteria gradually decreases. This has been proved time after time in the examination. The increase of the percentage of *B. acidophilus* is almost directly proportional to the decrease of that of *B. welchii*. In broth, the percentage of gas in the inner tube is greatly diminished, and in milk culture there is no stormy fermentation. The most striking change takes place in the Veillon tube in which there is a total disappearance of anaerobic gas formation characteristic of *B. welchii*. Microscopically, the fermentative flora present no typical morphology of *B. welchii*, and instead the fields are covered with gram-positive rods of the acidophilus group. In the intestinal flora *B. welchii* and *B. acidophilus* are the two antagonistic organisms, the presence of one is inimical to the other. When *B. welchii* is suppressed, *B. acidophilus* is the dominant organism to take its place. I agree with Meyer and others that in fermentative flora there is no *B. welchii* present. The theory of bacteriophage has been timely advanced by d'Herelle,⁷ and it is my belief that in some future time this interesting phenomenon can well be brought out in dealing with the relationship between these two organisms for the determination of intestinal flora.

I placed streptococci, diplococci and micrococci of any variety under the heading of gram-positive cocci, because they are very similar in their significance in the determination of flora. Torrey⁸ asserts that a high casein or milk diet stimulates the growth of saprophytic streptococci and diplococci. Among the streptococci present, the brevis type is the most prevalent. According to Andrew and Horder,⁹ this short type is *Streptococcus fecalis*, which ferments lactose, saccharose and mannite but not raffinose. The significance of this type in the intestinal flora has not yet been definitely brought out, but they often are present in a large number of diarrheal cases secondary to protozoan diseases, or any other intestinal disorder favorable for the production of diarrhea. The hemolytic type is rarely found in the stool. If present, it may be the result of metastasis due to focal infection in the upper respiratory tract. It is undeniable in diarrhea that the number of diplococci is very much

6 Herter. Common Bacterial Infection of Digestive Tract. New York, 1907.

7 D'Herelle. Bacteriophage, translated into English, 1922.

8 Torrey, J. C. J. M. Res. **39** 415 (Jan.) 1919.

9 Andrew and Horder. Lancet **2** 708, 1906.

increased, especially of the enterococci type described as *Micrococcus ovalis* by Thiercelin¹⁰ The number of diplococci is often increased in the arthritic cases secondary to focal infection, and in such the fields are often crowded with gram-positive diplococci, the number being approximately 90 per cent

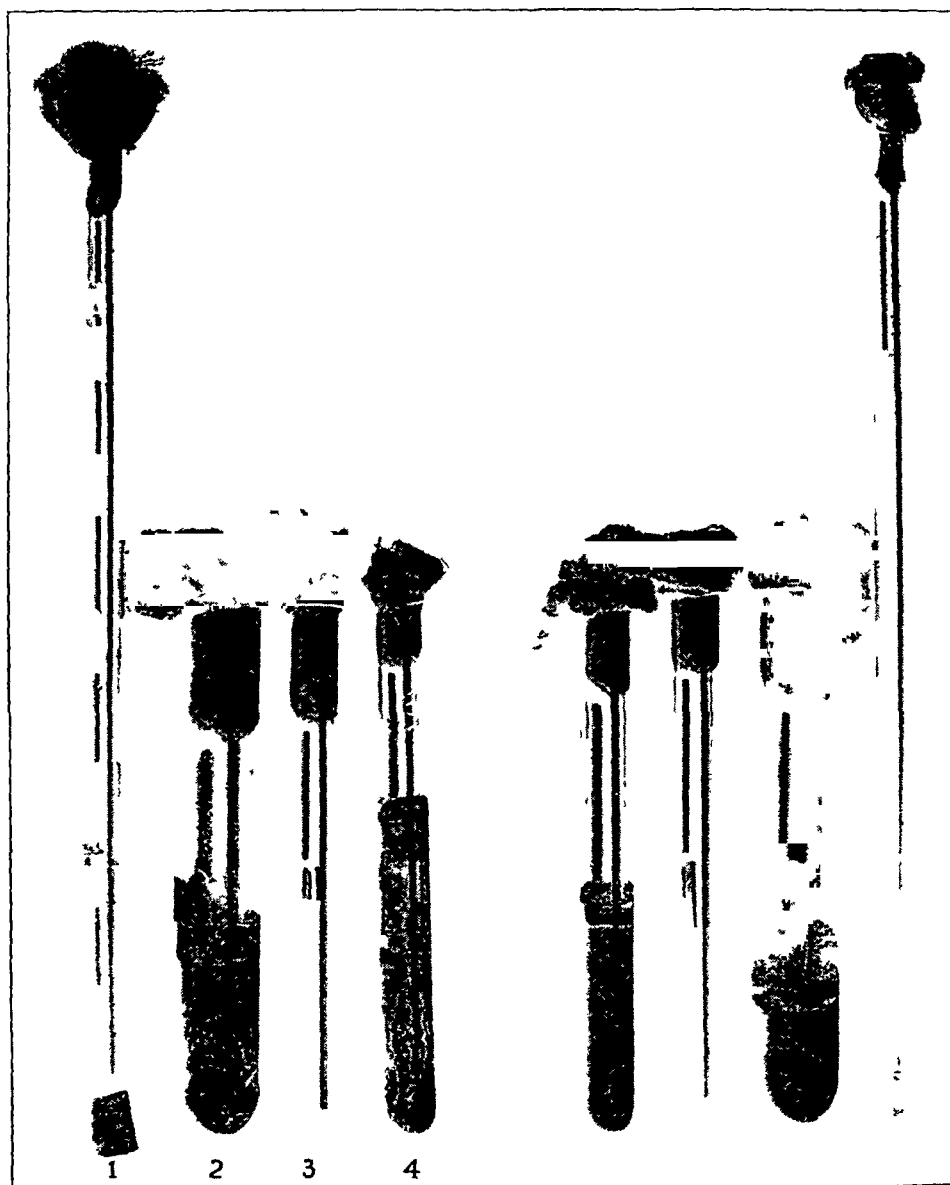


Fig 2—Cultural findings of intestinal flora, first group of tubes of putrefactive type, second of fermentative type 1, Veillon tube, 2, litmus milk, 3, 1 per cent lactose broth, and 4, milk for confirmatory test for *Bacillus welchii*

Yeasts do not bear any significance in interpreting the character of intestinal flora They are found as frequently in the putrefactive type as in the fermentative type So far as my experience goes with the

organism, there is no ground for the belief that the fermentative process is favored by their presence. They are often found in large numbers in a diarrheal stool. This, however, can be accounted for by an exaggerated peristalsis in these cases, appearing thus in the specimen without having undergone the destructive process in the digestive tract.

MACROSCOPIC AND CHEMICAL FINDINGS IN RELATION TO BACTERIAL ANALYSIS

The chapter will not be complete without touching on the rôles played by macroscopic and chemical findings in corroborating bacterial analysis. They often contribute more or less important data on the interpretation of the intestinal flora.

Amount of Stool—The amount of the stool has nothing to do with the types of intestinal flora, except in the case of constipation, especially of the atonic type. Here the quantity of the stool is much diminished owing to the excessive absorption by the intestinal mucus. The number of bacteria also is generally reduced, and the flora chiefly consists of the putrefactive bacteria. The aciduric type cannot exist in any obstructions along the intestinal tract favorable for chronic constipation.

Color—A dark brown suggests the putrefactive type of flora. The presence of this color is often characteristic of the meat regimen responsible for the condition. In green stools characteristic of the vegetable diet, putrefactive processes are very much lessened, while in the yellowish stools of the milk regimen the fermentative flora is often found.

Consistency—The scybalic stool harbors putrefactive bacteria. The fermentative type is mostly found in partly formed or mushy consistencies. Semiliquid and liquid types are not of desirable form so far as the character of the flora is concerned. In the latter, bacterial congregation is very numerous both in quality and quantity.

Odor—The odor of the stool has more to do with the interpretation of the flora than all other physical examinations put together. If the examiner accurately conducts his olfactory sense, the determination of flora is partially or wholly made before the bacteriologic examination is completed. The putrefactive type imparts an offensive odor owing to the presence of indol, skatol and mercaptan, particularly methyl mercaptan, as well as ammoma and hydrogen sulphid. The butyric acid odor is characterized by saccharobutyric fermentation in the flora dominated by *B. welchii*. The fermentative type is characterized by either sour or non-odor. The sour odor is due to the liberation of acetic and lactic acid by the activities of aciduric bacteria, which often partially or wholly inhibit the growth of purely alkaline or putrefactive bacteria. In a mixed type the odor varies according to the diet taken or the type

of bacteria dominant in the flora. Thus, in this type bacterial antagonism and symbiosis are at their height. The non-odor that often prevails in a putrefactive stool is probably due to excessive absorption of toxic substances by intestinal mucus, and the feces are usually scybalic. The reduction of the quantity of putrefactive by-products, therefore, means the betterment of the flora, and consequently that the odor of the stool is less offensive.

Reaction—The reaction varies according to the kinds of diet taken. In a strictly fruit regimen the reaction is acid, while in a meat regimen alkalinity prevails. In a carbohydrate regimen, the reaction is often acid. In my study, an acid reaction does not necessarily indicate a good flora, while the same can be said in regard to the alkalinity of the putrefactive flora. Robinson¹¹ concluded that the hydrogen ion concentration of the intestinal content has no relation to the type of the bacterial flora.

Mucus—The fermentative type is seldom found in the stool in which mucus is detected in large quantity. The presence of mucus always interferes with the process of transformation. Mucus is an albuminous substance, and favors the development of putrefactive bacteria. The presence of mucus, therefore, hinders the progress of the intestinal transformation. In cases of mucous colitis and other inflammatory and infectious diseases, mucus can be detected either separately or mixed with the stools. In those instances, the change of flora takes on a very slow course.

Blood—The presence of either occult or fresh blood suggests the inflammatory or infectious processes going on in the intestinal tract. It may be due to hemorrhoids, fissure or ulcer in the lower part of colon in case fresh blood is found, and ulcer or cancer of the gastro-enteric tract in case of occult blood. Any infectious processes, such as typhoid, tuberculosis or diseases of a parasitic nature, often result in the presence of fresh blood. In such a case transformation takes place very slowly, and in the majority of cases will never occur until the interference is removed.

Pathogenic Parasites—Pathogenic parasites are obstacles to the transformation of the flora. The changes that take place in the intestinal tract owing to their presence are not favorable for the growth and proliferation of *B. acidophilus*. In parasitic diseases, there is more or less a continual destruction of the intestinal wall, toxic substances are readily absorbed into the blood stream, and fermentative processes do not take place under such circumstances.

Summarizing these findings, we may say that the conditions favorable for the process of the transformation are a yellowish soft, fluffy

11 Robinson C. S. J. Biol. Chem. 52:445 (June) 1922

stool with sour or non-odor, and an absence of mucus and blood as well as of parasites regardless of reaction. On the other hand, if the stool shows scybalic or diarrheal types, is dark brown, of putrid odor, with or without mucus, blood and parasites regardless of reaction, the putrefactive process is evident provided the bacteriologic examination presents the equivalent picture.

CONCLUSION

1 The bacteriologic examination advanced here is not for a detailed study of the intestinal bacteria, but to determine the general character of the flora.

2 Litmus milk, lactose broth, Veillon tube and dextrose agar plate, together with special milk culture to determine the spore-bearing type and microscopic examination, constitute the analysis of the intestinal flora.

3 Macroscopic and chemical examinations are necessary to corroborate the bacterial analysis, but the test should be made independently.

4 The classification of the flora is an arbitrary affair. The interpretation should be made on the summarized findings of all the examinations concerned.

DEFECTS OF MEMBRANOUS BONES, EXOPHTHALMOS AND DIABETES INSIPIDUS

REPORT OF A CASE WITH NECROPSY ¹

CHESTER Q THOMPSON, M D

J JAY KEEGAN, M D

AND

A D DUNN, M D

OMAHA

In 1919, Christian ¹ under the title, "Defects in Membranous Bones, Exophthalmos and Diabetes Insipidus, an Unusual Syndrome of Dyspituitarism," reported a case presenting this syndrome and cited two similar cases previously described by Schuller ² Three other cases have since been reported by Hand ³ and one by Grosh and Stifel,⁴ making seven cases in all reported to date The following case was deemed worthy of a detailed report because of the unusually widespread osseous dissolution present, because of the rarity of the condition, and finally to record a complete postmortem study of this strange disease complex

REPORT OF CASE

History—C J, a boy, aged nine years, came to us, Feb 15, 1923, seeking relief from excessive thirst and frequent urination

The father, mother, one sister and one brother were living and well The mother had had no miscarriages One brother died when five months old of cholera infantum

The patient was born and raised in the Missouri Valley, his birth was at full term, the delivery being normal and his weight, 4.5 kg He was bottle fed from birth, lime water was not added to the feedings He had had chickenpox and scarlet fever in a mild form without complications His growth and development were normal

In December, 1922 (fourteen months before coming to us), the patient suffered a severe attack of measles which lasted for three weeks No complications fol-

* From the department of clinical investigation, University of Nebraska College of Medicine

1 Christian, H A Contributions to Medical and Biological Research (Osler Festschrift), New York, Paul B Hoeber, 1919, 1 390-401 As Christian's excellent paper has formed and will continue to form the basis for studies of this baffling syndrome, it is suggested that for the sake of simplicity the name Christian's syndrome be used temporarily instead of the cumbersome defects in membranous bones, exophthalmos and diabetes insipidus

2 Schuller, Artur Ueber eigenartige Schadeldefekte in Jugendalter-Fortschritte der Roentgenstrahlen, 1915-1916, 23 12

3 Hand, Alfred Defects of Membranous Bones, Exophthalmos and Polyuria in Childhood—Is It Dyspituitarism? Am J M Sc 162 509 (Oct) 1921

4 Grosh, L D, and Stifel, J L Defects in Membranous Bones, Diabetes Insipidus and Exophthalmos with Report of a Case, Arch Int Med 31 76 (Jan) 1923

lowed save slight looseness of the teeth and soreness of the gums, which was thought to presage the eruption of permanent teeth. Convalescence, however, was protracted and he was left in a weakened condition from which he never recovered. Six months after his measles, excessive thirst and urination suddenly appeared, persisted for several days, then disappeared. At this time a small soft spot was noted on the right side of his head, little attention was accorded this softened spot as the mother thought it to be "Nature's way of accommodating a growing brain." About one year after the measles (three months before coming to us), the polydipsia and polyuria returned and persisted with increasing severity until he was drinking at every chance and passing from 6 to 12 liters of urine daily. His strength began to wane and his appetite to fail. On several occasions he became nauseated and vomited. He had no headache and no visual disturbances.

Physical Examination—The patient was fairly well developed but emaciated. His color was sallow and dull and his facies that of an old man, but he was active, talkative and mentally keen, and apparently he felt well. The skin was



Fig 1—Patient, C J, six weeks before death, flattening of left side of skull, bulging of left frontal and parotid regions, exophthalmos (more marked on left), prognathism, absence of teeth and facies of an old man

coarse and dry. The chin was narrow and pointed, fulness was apparent in the left parotid region, and the frontal eminences were prominent. The skull was symmetrical without elevations. In the right parieto-occipital region was a soft, elastic depression about 4 cm in diameter, the edges of which were irregular, firm and sharply chiseled. There was no tenderness except to deep pressure, the hair was abundant, dry, coarse and brown, the scalp was clean and without scars. There was no discharge from the ears. The eyes were prominent but there was neither lid-lag nor extrinsic muscle paralysis. There was slight nystagmus in all directions. There was no photophobia, diplopia or lacrimation. The conjunctivae were normal, the sclerae were white, the pupils were large, equal and regular, reacting normally to light and distance, the fundi were normal, the vision was normal. There was no gross deformity of the nose and no evidence of obstruction or discharge. The hearing was normal. The lips were of good color without herpes, fissures or ulcerations. The mucous membrane of the mouth was normal but there was marked gingivitis. The teeth were clean and sound.

but loose. The tonsils were large and pale, liquid pus could be expressed from both. The pharynx was pale and covered with a slight amount of mucopurulent secretion. No pulsations were visible in the neck. The thyroid seemed small. Examination of the heart and lungs was negative. There was no evidence of an enlarged thymus. The abdomen was retracted and firm. There was no adenopathy. The hands were small and gracile. The genitalia were infantile. The deep and superficial reflexes were normal, there was no sensory disturbance or other evidence of peripheral or central nervous system involvement. The temperature was 36.6 degrees C, the pulse 90, the blood pressure was 90 systolic and 60 diastolic. The weight was 27.2 kg.

Laboratory and Special Findings—Hemoglobin totaled 75 per cent (Dare), erythrocytes, 4,120,000, leukocytes, 9,600, with normal spreads, the blood urea nitrogen was 19.5 mg and the blood sugar 50 mg per hundred cubic centimeters of blood, the blood Wassermann reaction was negative. The urine was water-pale and alkaline, the specific gravity was of 1.000, neither albumin, sugar reduction nor Bence-Jones albumose was found. Microscopic examination was negative. The cerebrospinal fluid was clear and came under normal pressure. The gold solution curve was 0000000000, globulin was slightly positive, there were four cells per cubic millimeter, the Wassermann test was negative. The spinal puncture had no effect on the water output. The basal metabolic rate was plus 8.

Roentgenograms taken at this time showed the skull (Fig 2) to be normal in size and shape, with smooth and regular surfaces except at the vertex and in the frontal regions where the outer table alone was lacking. The thickness of the bones was otherwise normal. There appeared to be no diffuse involvement of bone, but throughout the upper three-fourths of the cranium were scattered numerous defects varying in size, roughly, from 2 to 40 mm in diameter. The smaller areas were round or oval while the larger ones were quite irregular. The distribution of the lesions was asymmetrical. The superior orbital plates were involved. Both maxillae showed spotted areas of bone absorption similar to those in the skull. The sella turcica appeared normal in size and shape, its floor smooth and regular. It measured 12 by 8 mm. The anterior and posterior clinoid processes were 5 mm apart. The pelvic bones showed lesions similar to those in the skull. In the left ilium was an irregular area 15 by 20 mm. The right ilium, as well as both ischia, showed smaller scattered areas of rarefaction (Fig 3). The right femur showed beginning lesions only in the upper part of the shaft, but in the upper third of the shaft of the left femur and in the neck there were definite areas of rarefaction. The epiphyses on the heads and greater trochanters were ununited. Partial collapse of the body of the fourth lumbar vertebra was evident. At this time a tentative diagnosis of multiple myeloma was made from the roentgenologic findings, even in the absence of Bence-Jones albumose and of all evidence of tumor.

Interval History—Subcutaneous injections of pituitary extract accomplished a reduction of the urine output of from 4 to 8 liters a day. The specific gravity

EXPLANATION OF PLATE 1

Fig 2—Skull, Feb 15, 1923, nine months after the first appearance of symptoms

Fig 3—Pelvis, Feb 15, 1923, showing definite bone defects in ilia, ischia and femur

Fig 4—Skull, Feb 14, 1924, one year later than Figure 2

Fig 5—Skull, June 21, 1924, marked increase in bone destruction, which had taken place in four months

Fig 6—Pelvis, June 21, 1924, increase in bone destruction

Fig 7—Chest showing bone defects in humeri, clavicles and ribs. The fine mottling of an interstitial pulmonitis that was characteristic in stereoscopic plates can be detected.



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varied from 1 000 to 1 010 After six months of hypodermic pituitary extract treatment, epistaxis set in, the patient's appetite failed, and each injection of pituitary extract was followed by pain in the epigastrium and precordial distress The pituitary extract was discontinued The distressing abdominal symptoms were relieved but the polyuria returned with increasing severity The patient was given 45 grains (29 gm) of calcium lactate daily, a sufficient quantity of lime water in milk, and ground egg-shells in capsules (a home suggestion) The taking of lime salts apparently affected the boy's sense of well-being because when they were stopped on several occasions he remarked that he did not feel so well Thyroid therapy was of no apparent benefit A nasal spray of pituitary extract controlled the polyuria quite satisfactorily About eight months after the first examination exophthalmos was already marked and lacrimation troublesome He suffered some from lumbar backache and slight pain in the left hip at times, but he had always been about and felt quite well Except for a troublesome cough with a mucopurulent sputum, which was repeatedly negative for tubercle bacilli, and an ominously increasing dyspnea, the patient's general condition remained surprisingly good

The following notes were taken from an examination record made June 21, 1924, about two years after the onset of the disease, sixteen months after our first observation and six weeks prior to the patient's death The patient walked with a marked limp, listing forward and to the left in the manner of a congenital dislocation of the hip Pallor was pronounced, the cheeks were sunken (there

Calcium Determination

Urine	Amount	Method	Calcium
7/ 9/24	2,600 c c (24 hours)	McCrudden	128 mg
	2,600 c c (24 hours)	Kramer and Tisdall	152 mg
7/10/24	1,400 c c (12 hours)	McCrudden	8 mg
	1,400 c c (12 hours)	Kramer and Tisdall	36 mg
7/15/24	3,440 c c (24 hours)	McCrudden	73 mg
Blood Serum			
7/15/24	13.6 mg per 100 c c		

had been a loss of 27 kg in body weight), the eyes were prominent, and the palpebral fissures widened (Fig 1) Exophthalmos was marked, lid-lag was present, and the visual fields were normal Fulness in the left parotid region and bulging of the forehead between the eyes was striking The entire skull was soft and doughy with only a coarse irregular framework of supporting bony ridges Straining or coughing caused the soft areas to bulge The gingivitis was severe and the teeth were held only by the soft investing tissue, as the alveolar processes had been absorbed There was some mucopurulent material on the posterior pharyngeal walls and pus could be expressed from the tonsils and gums There was moderate general adenopathy, which had developed during the last year, but the spleen was not enlarged The lungs were hyperresonant to percussion with numerous small and large dry râles and prolonged expiration The spine had become bent forward in the lumbodorsal region with an even curve There were no visible or palpable bony deformities of the extremities or spine

The laboratory findings at this time were hemoglobin, 60 per cent, erythrocytes 3,800,000, leukocytes, 10,200, with polymorphonuclears, 71 per cent, small lymphocytes, 20 per cent, large lymphocytes, 18 per cent, and eosinophils, 1 per cent The urine was clear and alkaline in reaction with a specific gravity of 1 000 without albumin, sugar or Bence-Jones albumose Microscopic examination was negative The calcium determinations are given in the accompanying table

5 The methods used were those of McCrudden, F H J Biol Chem 7 83, 1910 and of Kramer, B. and Tisdall, F T Bull Johns Hopkins Hosp 32 44 (Feb) 1921 It is to be regretted that hospitalization was not permitted for complete metabolic studies as a satisfactory solution of this syndrome will probably not be obtained without more complete knowledge of its metabolism

A roentgenologic study of the entire skeleton at this time revealed the following

The skull truly merited the term geographic (Fig 5). On the left side of the skull in the rear portion of the left frontal bone there was an irregular area of bone estimated at 30 mm long and 20 mm wide, and an irregular area in the parietal bone 20 by 15 mm. Except for these islets of bone the entire frontoparietal region was gone. The occipital bone was largely free from destruction except in the region of the lambdoid suture. On the right side easily half the skull was gone and on the left side fully three-fourths. There was a definite dissolution of continuity, or *faulting* of the frontal bone, measuring fully 4 mm. The sella turcica, except for a small spot in the anterior wall, was without evidence of erosion and was normal in shape and contour except that the posterior clinoid processes were quite thick, set well back and erect. The sum total of bone loss in the skull had practically doubled in the last five months. In one and one-half years it had increased at least tenfold (Figs 2, 4 and 5). Decalcification was extensive in both jaws.

The bones in both feet and legs were normal except that the shafts of the tibiae showed a moderate periostitis. The bones of the hands and forearms were normal.

The ilia showed many and extensive areas of decalcification (Fig 6). The upper end of the shaft and the neck of the left femur showed one decalcified area about 2 cm in diameter. The right ischium showed several areas of decalcification and also some excess of bone production. Practically all the ribs showed areas of decalcification. The shaft of the right humerus throughout its middle third showed numerous small areas and the head of the left beginning spots of decalcification (Fig 7). The cervical vertebrae showed considerable decalcification which was especially noticeable in the sixth and seventh. There were bone defects in both scapulae. The clavicles appeared normal.

Roentgenograms of the chest (Fig 7) revealed the diffuse increase in density characteristic of chronic interstitial pulmonitis.

As death approached the cough and dyspnea became more and more troublesome. Attacks of nocturnal dyspnea with tachycardia recurred with increasing frequency. Exitus came quite rapidly from cardiac failure, attributed to impaired circulation incident to extensive pulmonary fibrosis.

CLINICAL COMMENT

It is obvious that clinical interest in this unusual syndrome must center about (1) the bone destruction and the mechanical disturbance that may arise therefrom, (2) the sella turcica because of prevalent conception of the rôle of the hypophysis in skeletal growth and diabetes insipidus, (3) the pathogenesis of diabetes insipidus, and (4) any etiologic inferences the behavior of the disease may suggest.

Defects in Bones—In surveying the cases reported, "defects in membranous bones" loom large. The defects in the cranial bones in our case made a startling picture—a head of jelly with only a few struts and guides of bone to support it. The *faulting* of the frontal bone with its orbital plates fully explained the exophthalmos. After the patient had been up and about, the exophthalmos was relatively equal in both eyes, after he had lain for an hour or two on either side the protrusion of the "down" eye would increase markedly and the "up" eye would recede. Physiologic disturbances in other intracranial structures, such as the hypothalamus, might be logically postulated on a

mechanical basis alone, or through involvement of adjacent tissues by the same processes that were accomplishing bone destruction. The peculiar gait and "listing" of our patient was accounted for by the destruction of the body of the fourth lumbar vertebra.

Sella Turcica—Changes in the sella have not been an outstanding feature in reported cases.

The sella turcica was somewhat enlarged and slightly flattened and in the stereoscopic view it looks as if there is a slight defect in the base of the skull very close to, if not continuous with, the sella turcica (Christian).

The sella turcica is markedly changed, only the dorsum sella is present, the anterior part of the floor of the sella is greatly deepened (Schuller's Case 2).⁶

In only one of Hand's cases was there a roentgenogram, this showed "the sella turcica to be uninvolved in the bone change." In Grosh and Stifel's case, they stated that the bones forming the bridge of the nose were not involved nor the sella turcica, which was normal. In our case stereoscopic roentgenograms revealed no changes in the size or contour of the sella such as might reasonably denote abnormalities in the hypophysis, only a small defect in the floor of the sella being suspected. Out of five cases in which mention of the sella was made only one case showed definite sellar changes, a high percentage of normality if we are to assume a pituitary origin for the syndrome. Furthermore, because the hypophysis seems to have something to do with the laying down of bone in skeletal growth there is no evidence that by a reverse process it may cause wholesale destruction of disseminated areas of normally built bone.

Diabetes Insipidus—While diabetes insipidus has been commonly found in lesions of the pituitary gland, two views prevail as to its pathogenesis. An older and generally accepted view is that it is due to pituitary disease. Rowntree⁷ was unable to find in the literature any case of diabetes insipidus in which the necropsy failed to show changes in the pituitary gland. Motzfeldt⁸ concurs. Engelbach and Tierney⁹ also assume a deficiency disease and call attention to the "strange tendency to exclude pituitary disorder in the absence of typical sellar erosions or deformities." Furthermore, that posterior lobe extract or pituitary extract is the only agent known to control the polyuria is interpreted in

6 It would seem that for the sake of clarity Schuller's Case 1 should not be included in this syndrome. This patient did not have diabetes insipidus but dystrophia adiposo genitalis, the defect in the cranial bones was largely confined to one side (the left). Schuller's assumption was a tumor at the base of the brain ("other changes suggested pressure"), either an angioma of the dura or of the skull.

7 Rowntree, L. G. *Oxford Med* 4 185, 1921.

8 Motzfeldt, K. *Endocrinology* 2 94, 1918.

9 Engelbach, W., and Tierney, J. L. *Pituitary Polyuria, Internat Clinics* 4 123, 1920.

the light of substitution therapy. On the other hand there is much experimental evidence for the other view, that diabetes insipidus is of extrahypophyseal, or basilar, origin. Sajous¹⁰ gives an excellent exposition of this theory. The argument that diabetes insipidus is a pituitary deficiency disease because the polyuria is controlled by pituitary extract is met by the argument that no one would assume that asthma is a suprarenal deficiency disease because it may be controlled by epinephrin. Furthermore, there is much evidence, largely experimental, in favor of an extrahypophyseal origin. Camus¹¹ and Roussy¹² punctured through the sphenoid bone with a heated drill without injuring the hypophysis, the region known to anatomists as the hypothalamus and thus induced permanent polyuria. They also produced polyuria in a dog without polyuria from which the hypophysis had previously been removed, demonstrating that the polyuria did not depend on the hypophysis. Bailey and Bremer¹³ repeated the experiments and confirmed the results of Camus and Roussy, using the Paulesco-Cushing method of lateral approach. They succeeded in producing polyuria in thirteen out of thirteen dogs by injury of the hypothalamic region without trauma to the hypophysis. The results of numerous tests carried out on these dogs justified to them "the conclusion that the persistent polyuria provoked in this manner (by lesions of the para-infundibular region of the hypothalamus) has all the characteristics of diabetes insipidus." This experimental diabetes persisted after denervation of the kidney and therefore could not be attributed to nervous or vasomotor disturbances. Hanchett¹⁴ was able to produce polyuria in only one instance in nine dogs by stimulation of the anterior and posterior lobes of the hypophysis with a hot rod. On the other hand he was able to induce polyuria regularly by traction on the stalk. Hanchett concludes

Experimental lesions of the hypophysis similar to those producing negligible excretory changes, when associated with traction on its attachment to the floor of the third ventricle, uniformly produced polyuria. The degree of polyuria was roughly in proportion to the amount of traction.

Curtis¹⁵ confirmed the experimental results of Camus and Roussy, Hanchett, and Bailey and Bremer in that diabetes insipidus is essentially of hypothalamic origin, he regards experimental diabetes insipidus

10 Sajous, C. E. D. *Am J M Sc* **167** 679-696 (May) 1924

11 Camus, J., and Roussy, G. *Compt rend Soc de biol* **75** 483, 1913

12 Camus, J., and Roussy, G. *Endocrinology* **4** 507 (Oct-Dec) 1920

13 Bailey, Percival, and Bremer, Frederic. *Experimental Diabetes Insipidus*, *Arch Int Med* **28** 773 (Dec) 1921

14 Hanchett, M. *Experimental Polyuria*, *Am J M Sc* **163** 685 (May) 1922

15 Curtis, G. M. *The Production of Experimental Diabetes Insipidus*, *Arch Int Med* **34** 801 (Dec) 1924

as essentially a "hypothalamic thirst phenomenon" In the absence of definite sella changes and in the light of recent experimental results we were not justified in assuming clinically the existence of hypophyseal disease in our patient

Etiologic Inferences—The close relationship of antecedent infections to the disease is worthy of notice Christian's patient had mumps four months before entrance to the hospital, and loosening of the teeth and swelling of the gums six months before In Schuller's Case 2, the patient had whooping cough six months prior to the sudden development of left sided exophthalmos Hand's first case was admittedly infectious and was diagnosed tuberculosis, although the necropsy failed to demonstrate characteristic tuberculous lesions In Hand's Case 2, recession of the gums with the gradual loss of teeth began from five to six months after scarlet fever At the same time a soft spot was noted in the head Twenty months after the scarlet fever, diabetes insipidus appeared¹⁶ In Grosh and Stifel's case polyuria developed seven weeks after drainage of the left mastoid, preceding this for some time the patient had had a discharging ear Our patient never recovered fully from a severe attack of measles followed by sore gums and loosening of the teeth, which preceded the first attack of polyuria and the first softening of the bone by six months In only three cases were leukocyte counts made In Christian's case, the leukocyte count ranged from 6,600 to 12,600 with an occasional rise to 14,100, 15,600 and 20,100, the differential on admission showing polymorphonuclears 30 per cent, lymphocytes 45 per cent, large mononuclears 23 per cent and eosinophils 2 per cent In Grosh and Stifel's case, one count of 8,000 leukocytes with normal spreads is recorded In our case, three counts varied from 9,600 to 10,200, with polymorphonuclears 71 per cent, small mononuclears 20 per cent, large mononuclears 18 per cent and eosinophils 1 per cent on one count The temperature was not noted in any of the other cases, except in Hand's first case, in which temperatures of 102.2, 99 and 105 degrees were noted Occasional unaccountable rises of temperature to 101 degrees were noted in our patient The existence of widespread infection was further supported by the signs of diffuse pulmonary infection with the clinical and roentgenologic findings of a chronic interstitial pneumonitis, infected tonsils, gingivitis, postnasal discharge and the later development of a general adenopathy

NECROPSY

The body was that of an embalmed white boy, appearing older than the given age of 11 years The development was fairly normal, the skin was pale and nutrition poor

16 In Hand's Case 3, which was incompletely studied, there was no polyuria, but the patient did have characteristic defects in the membranous bones

In the head, the most noticeable feature was a marked bilateral exophthalmos, more pronounced in the left eye, which was distinctly lower than the right (estimated 5 mm). The nose was small and the bridge sunken. Many teeth were absent. The contour of the cranial vault was distinctly asymmetrical with a soft flattened forehead and soft, pliable parietal regions. Palpation revealed an extensive bone deficiency—a membranous cranium. A narrow, irregular, sharply delimited bridge of bone extended diagonally across the vault from the right external orbital process to the left mastoid. In the frontal region, the membranous cranium was limited by a sharp border with two processes jutting upward about 3 cm from each supra-orbital ridge. In the midline the bone deficiency extended to and included the nasal bones. In the left temporal region, the membranous cranium reached to the level of the zygomatic arch, while in the right temporal region, the bone border was at the middle of the squamous part of the temporal bone. Posteriorly, the bone deficiency was limited by the superior occipital line, except for a projecting spine of bone about 2 cm long in the midline. The scalp and aponeurosis stripped easily from the bone and the membranous cranium beneath. In the center of the largest membranous areas, there was slight adherence by yellowish fibrous tissue. The underlying membranous cranium was opaque and had a mottled coagulated appearance. The bone edges were abrupt and sharp.

The membranous cranium, which included a rather firmly adherent dura mater, was cut easily with knife and scissors. The subdural space was normally free. The inner surface of the dura beneath the membranous cranium was mottled by a dull yellowish tissue which took a scarlet and fat stain. Microscopic section through this region showed a lining layer of large oval cells, with sharp borders, with clear, slightly granular cytoplasm, and small compact centrally placed nuclei. The cytoplasm contained a variable amount of lipoid material in finely divided form. The cranial membrane that this fatty layer lined consisted of typical dense white fibrous tissue, with no evident lines of cleavage between the dura proper, the endosteum, the absorbed cranial bone and the periosteum. The dura of the falx cerebri and that beneath the intact cranial bone had a normal smooth, white, glistening appearance and stripped easily, revealing a gray, translucent tissue fitting closely into the groove between the two tables at the bone margin. Microscopic sections of this tissue at the bone edge showed a highly cellular tissue composed chiefly of large cells with eosinophilic cytoplasm and large reticular nuclei. In many places, particularly at the bone border, these cells appeared to have fused to form large multinuclear, foreign body giant cells (Fig 8, giant cells at bone border). The nuclei in some were arranged peripherally in a semicircular manner, but the majority were centrally located, the nuclei numbering from a few to twenty or more. The remaining cells of this soft tissue consisted of lymphocytes, plasma cells and polymorphonuclear leukocytes and eosinophilic cells. Quite dense infiltrations of each of these cell types could be found in different regions. There was a gradual narrowing and complete fibrosis of this tissue from 5 to 10 mm from the bone edge, where it joined the dura to form the membranous cranium (Fig 9, transition from cranial bone to membrane). The larger areas of bone by transmitted light showed round or oval decalcified areas of variable size, bounded by the same grayish cellular tissue at the border. The dura and periosteum were adherent over these areas. Other darkened areas were seen in the bone where the external and internal tables were intact and the periosteum not firmly adherent. Microscopic section of these showed a beginning cellular proliferation and infiltration in the diploetic spaces similar to those described above.

The exposed convex surface of the cerebral hemispheres appeared entirely normal in size, color and conformation. There was no involvement or thickening of the pia-arachnoid. Elevation of the frontal lobes of the brain revealed an enlarged, yellowish infundibular stalk, measuring from 2 to 3 mm in diameter. This yellowish involvement of the infundibulum extended as a

bulbous enlargement into the tuber cinereum (Fig 10, basilar view of tuber cinereum) and was distinctly outlined in the median sagittal section from the optic chiasma in front and the gray brain substance above (Fig 11, median sagittal view of tuber cinereum) Serial sections were made of the tuber cinereum and adjacent structures (Fig 12, sagittal section of tuber cinereum) The region of the pathologic condition appeared as a darker stained area 3 mm in diameter, with a somewhat indistinct upper border, which was histologically similar to the soft tissue at the cranial bone edge At the lower edge of the infundibulum, several normal glandular acini were seen surrounded by a millimeter or two of tissue composed chiefly of large cells with abundant pale eosinophilic cytoplasm and fairly large reticular nuclei A few cells appeared to have fused to form multinuclear giant cells There was a moderate infiltration with mononuclear cells, but with very few polymorphonuclear leukocytes A process of fibrosis appeared under way, the cells alining themselves in parallels and whorls and having the appearance of fibroblasts There were very few blood vessels The microscopic picture farther up into the tuber cinereum showed a central strand of dense white fibrous tissue, continuous with a very dense fibrosis comprising the entire area of junction with the nervous tissue (Fig 13, dense fibrosis of tuber cinereum) Where this pathologic condition extended into the brain tissue, there was a marked perivascular mononuclear cell infiltration This perivascular infiltration extended a short distance into the nervous tissue beyond the fibrosis (Fig 14, perivascular infiltration in nervous tissue) Bordering on the dense fibrous tissue of the tuber cinereum was the characteristic eosinophilic cellular tissue described in the infundibulum This extended several millimeters upward along the posterior border of the tuber cinereum, apparantly as a pial perivascular infiltration, to the same level as the perivascular infiltration in the nervous tissue Multinuclear giant cell formation was quite prominent in this region (Fig 15, multinuclear giant cells in the tuber cinereum) A focus of dense small mononuclear cell infiltration was present at the base of the fibrous one (Fig 16, focus of lymphocytic infiltration in tuber cinereum) There was a sharp line of separation anteriorly between the optic chiasm and the cellular zone A slight perivascular cell infiltration extended into the upper and lateral part of the chiasm

The hypophysis with its dural envelop was removed intact. It was of normal size and reddish The infundibulum at its junction with the gland was of normal size and appearance, measuring from 1 to 2 mm in diameter A gross sagittal section of the pituitary gland showed normal anterior and posterior lobes Serial sagittal sections of the gland and dura, including the infundibulum, were made Microscopically, the anterior lobe showed the normal elements of large, brightly stained pink cells (eosinophilic), large, darkly stained blue cells (basophilic) and a few small, pale blue cells (neutrophilic or chromophobic) arranged in cords and separated by vascular stroma The basophilic cell predominated in the anterior region where there were only scattered eosinophilic cords The eosinophilic cells predominated in the central region

EXPLANATION OF PLATE 2

Fig 8—Foreign body giant cells, polymorphonuclear leukocytes and plasma cells in tissue obtained from seat of active bone destruction

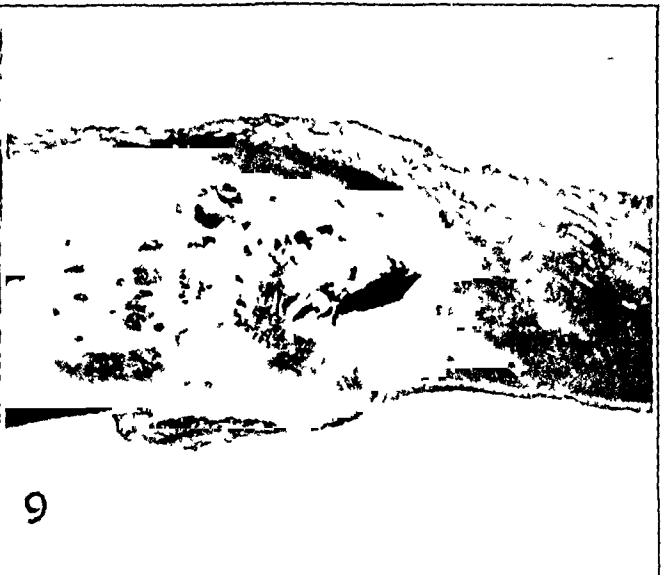
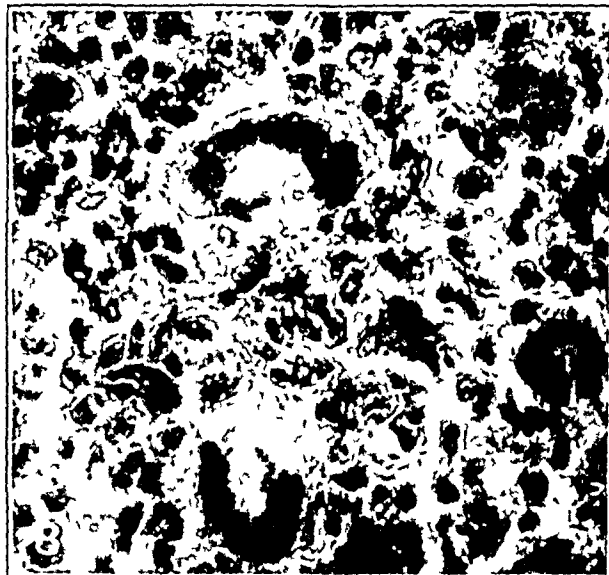
Fig 9—Normal bone showing area of active bone destruction with fusion of periosteum, endosteum and dura to form the membranous cranium

Fig 10—Basilar view of tuber cinereum with yellowish bulbous enlargement

Fig 11—Median sagittal view of tuber cinereum

Fig 12—Sagittal section of tuber cinereum showing area of active inflammation

Fig 13—Dense fibrosis in tuber cinereum



and all three types of cells appeared indiscriminately mixed in the posterior region, with possibly a predominance of neutrophils (chromophobes). The pars intermedia was rather poorly defined but was identified by its position and by its fibrous stroma, indefinite cell elements and a few alveoli containing colloid. The pars posterior or pars nervosa was not entirely normal. The most evident pathologic condition was a slight infiltration of polymorphonuclear neutrophilic and eosinophilic leukocytes without increase of fibrous tissue. Small scattered foci of degenerated cells and peripheral foci of basophilic mononuclear cells resembling lymphoid follicles were found. The pathology was interpreted as a subacute degenerative and inflammatory process of relatively recent origin. The base of the infundibulum showed a considerable dense, white fibrous stroma with an extension into it of the normal cellular elements of the pars posterior and of the glandular elements of the pars anterior. Surrounding this was a tissue composed chiefly of faintly stained large eosinophilic cells, fusing in places to form multinuclear giant cells, and moderately infiltrated with smaller mononuclear cells. This tissue, which had embedded in it a few glandular cords and alveoli, was thickest on the anterior surface of the infundibulum and could be traced a short distance into the superior portion of the pars anterior of the pituitary gland. The dura surrounding the pituitary gland appeared intact as a dense, white, fibrous tissue, although anteriorly there was attached to its outer surface a cellular tissue composed of pale eosinophilic cells and a varying amount of fibrous stroma, infiltrated with mononuclear, polymorphonuclear and eosinophilic cells. This tissue evidently arose from an adjacent decalcified area of bone in the right anterior wall of the pituitary fossa. It did not appear to extend through the dura at any place. Posteriorly, the dura surrounded a rather narrow extension of the pars posterior, the center of which showed some necrosis. Above the pars posterior there was quite a thick fibrous layer which was continuous with the fibrous septum of the pars intermedia and also with the central fibrous strand of the infundibulum.

The pituitary fossa appeared of normal size and shape except that the anterior clinoid processes seemed to be depressed, owing to a dropping of the lesser wings of the sphenoid bone and the superior orbital plates. The following measurements of the pituitary fossa were taken between anterior clinoids: 23 mm, anterior-posterior, 12 mm, width of dorsum sellae, 17 mm, thickness of dorsum sellae, 4 mm, depth of pituitary fossa, 9 mm, width of base of pituitary fossa, 11 mm, and anterior-posterior of fossa, 10 mm. In the right anterior and inferior region of the pituitary fossa was a decalcified area of bone, 5 mm wide and 8 mm long, which extended forward under the lesser wing of the sphenoid (Fig 17, pituitary fossa showing erosion in the floor). The floor of the skull showed extensive bone defects in the anterior and middle fossae. Bone destruction was so distributed in the anterior fossa that the entire mesial borders of the superior orbital plates were freed and could be easily depressed with the finger. The left orbital plate was loosened by extensive bone absorption which had involved the temporal plate of the frontal bone, the greater wing of the sphenoid, the squamous portion of the temporal bone

EXPLANATION OF PLATE 3

Fig 14—Perivascular infiltration in brain tissue adjacent to tuber cinereum

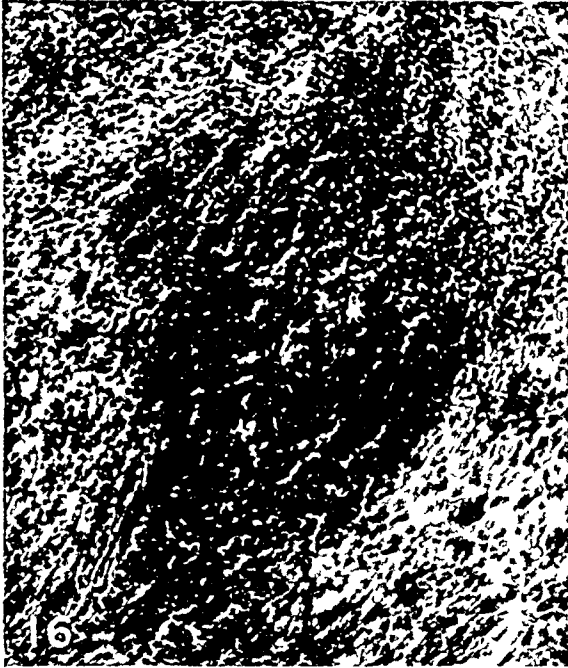
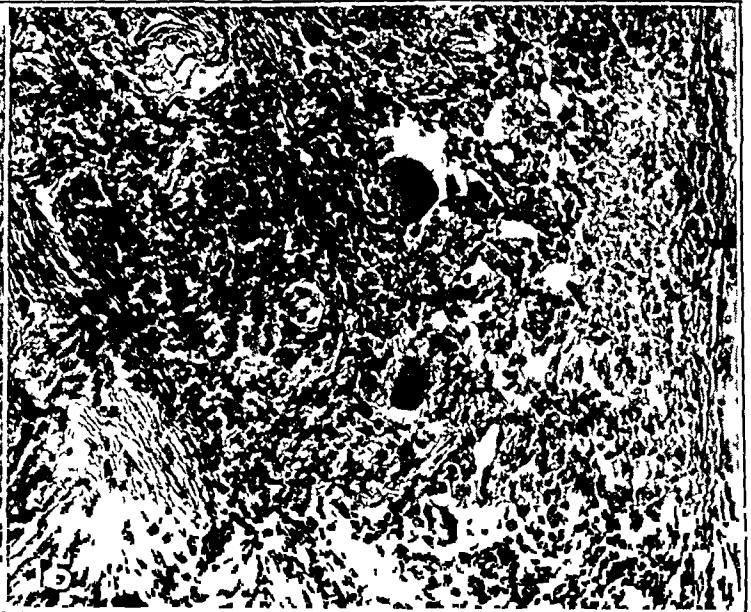
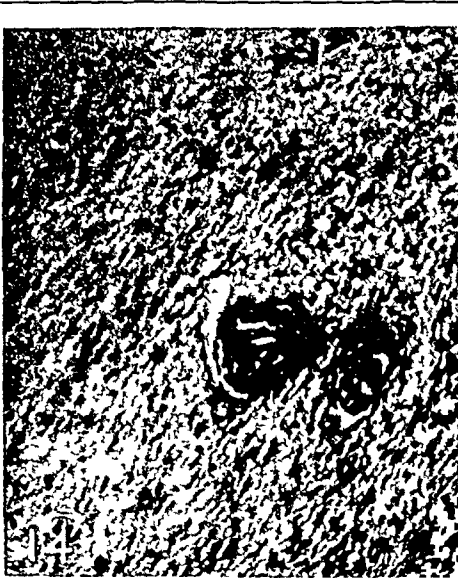
Fig 15—Multinuclear giant cells in tuber cinereum of same type as found at bone margin

Fig 16—Focus of lymphocytic infiltration in tuber cinereum

Fig 17—Bone defects in base of skull, showing faulting of orbital plates

Fig 18—Proximal end of left femur showing bone defects

Fig 19—Interstitial fibrosis of lung, the same general process as found in the bone margin and tuber cinereum



and the suture line between the orbital plate and the lesser wing of the sphenoid. Some supporting bone remained on the right side. The loosening and depression of the superior orbital plates could easily account for the exophthalmos.

The greater wings of the sphenoid bone were extensively decalcified in an irregular manner. The larger defects were filled with a mixture of dense yellowish-white fibrous tissue with grayish cellular tissue at the bone margins. Sections of this tissue showed the same cellular characteristics as described in the cranial bones of the vault. Mononuclear cells predominated, with scattered giant cells, and polymorphonuclear and eosinophilic cell infiltrations. The white fibrous tissue showed dense collagen fibrils and a few small nuclei. The squamous portions of both temporal bones were decalcified in a similar manner; there was no involvement of the petrous portions, mastoids, or middle ear cavities. The nuchal portion of the occipital bone was intact but most of the cerebral portion was decalcified. The sphenoid sinus was involved in the destructive process affecting the anterior cerebral fossa and was filled by dense white fibrous tissue. The ethmoid cells were likewise obliterated by tissue growth.

The proximal extremity of the left femur was removed and examined. A round decalcified area, 1 cm in diameter, was found on the lateral surface just below the greater trochanter (Fig 18, proximal extremity of the left femur). There was no change in contour lines, no unusual adherence of the periosteum, and the bone edge surrounding the area was quite sharp. The same process involved the cancellous bone between the trochanters. The cut surface showed a fairly firm, grayish white tissue. Sections of this tissue, including the bone border of the lateral cortical defect, showed the same process of variable cellular infiltration, multinuclear giant cell formation and transformation from loose to firm fibrous tissue described elsewhere.

The thyroid gland was of normal size, appearance and histologic structure. Two parathyroid glands were identified in the posterior capsule of the thyroid as small grayish red oval bodies about 2 mm long; they were histologically normal.

The pleural cavities were free from adhesions. The lungs were firm and pale from formaldehyd fixation. No nodules were present. Microscopically, there was found an extreme interstitial fibrosis, the alveolar walls being thickened to a degree which almost obliterated the normal lung architecture (Fig 19, interstitial fibrosis of lung). There was a moderate mononuclear cell infiltration in this tissue and numerous small blood vessels. A few polymorphonuclear cells were present. Some of these thickened septums were covered with a single layer of cuboidal epithelium. In places this appeared desquamated and formed confluent masses of cells. The bronchioles were enlarged and lined with a hyperplastic layer of columnar epithelium. There was no cellular material in the lumina. The heart was slightly enlarged, the right ventricular wall particularly was thickened. The valves were normal. Microscopically, the myocardial fibers were well defined, slightly granular, closely approximated and not fragmented. There was no infiltration. The aorta and coronary vessels were normal.

When the abdomen was examined, the peritoneum was smooth and glistening. The liver was about 5 cm below the costal margin in the midclavicular line, dark red and enlarged. Microscopically, the liver cords were everywhere well defined, the cells showing little granular degeneration or vacuolation. A few cords contained bile pigment. The liver sinuses were dilated and contained an occasional polymorphonuclear leukocyte. The portal areas showed no fibrosis. The gallbladder was normal and the bile ducts patent. The stomach was contracted, pale and showed no tumor masses or ulcers. Grossly and microscopically, the gastro-intestinal tract was practically normal. The pancreas was grossly and microscopically normal. The spleen was slightly

enlarged, firm and dark red. Microscopically, there was a marked engorgement of the sinuses with red blood cells and a considerable number of cells packed with hemosiderin pigment. The septums were not thickened. The splenic nodules were quite prominent as basophilic cellular areas as contrasted with the eosinophilic pulp. The central arteries were normal. The kidneys were distinctly enlarged and slightly lobulated. Gross section showed a normally striated and delimited cortex, 7 mm in width. Microscopic sections showed numerous large normal glomeruli. The convoluted tubules had large cells with abundant eosinophilic granular cytoplasm, brush border and slight elevation into the lumen. The loops of Henle could be characteristically differentiated into descending and ascending limbs. There was no interstitial tissue increase or infiltration. The pelvis of the kidney and the ureters were normal. The bladder wall was markedly thickened from muscle hypertrophy. The mucosa was pale and smooth. The suprarenals were of normal size, the cortex yellow, the medulla intact and not thickened. They were histologically normal.

The pathologic diagnosis was exophthalmos, decalcification of the bones, fibrosis of the tuber cinereum, slight subacute inflammation of the pars posterior of the pituitary gland, chronic interstitial fibrosis of the lungs, hypertrophy of the heart, chronic passive congestion of the liver and the spleen, hypertrophy of the kidneys, and hypertrophy of the bladder.

A necropsy of a case of defects in membranous bones, exophthalmos and diabetes insipidus, while giving highly instructive and suggestive findings, fails to fix beyond all peradventure the nature and cause of the disease. Histopathologic studies point to an inflammatory rather than to a degenerative or a primary metabolic process. The absence of a material pathologic condition in the anterior lobe of the hypophysis excludes it as a factor in the bony defects. The relatively negligible changes found in the pars posterior and the fact that their apparent origin was more recent than those changes found in the tuber cinereum, in the bones, and in the lungs eliminates the pars posterior as an important factor in pathogenesis. If the hypophysis were to be held in any way responsible for such widespread, bizarre disease processes one would expect more than the rather negligible pathologic changes found in the gland proper. The other ductless glands were found to be normal.¹⁷ The preponderance of evidence is against an endocrinologic origin for the syndrome unless perchance one wishes to consider the tuber cinereum an endocrine, and to attribute to a diseased tuber unlimited powers of bone destruction. The changes in the stalk appeared of more recent origin, lessening the possibility of its participation in the causation of the syndrome. That the lesion in the tuber cinereum was the probable cause of the diabetes insipidus, however, is supported by the results obtained by Camus and Roussy, Hachett, Bailey and Bremer, and Curtis in their work on experimental polyuria in dogs. The lesions in the tuber cinereum were of the same character and apparent maturity as the lesions in the bone and in the lungs, which suggests that these

17 While the testes were examined at necropsy and were pronounced grossly normal, they were inadvertently not included in the material saved for histologic studies.

lesions were produced by the same agent at about the same time. The inflammatory character of the histopathologic condition, supported by numerous facts brought out in the clinical study, viz, fever, onset subsequent to measles, secondary anemia, chronic interstitial pulmonitis, marked gingivitis, markedly infected tonsils, etc., points to an infectious origin. In other words, an organism with bone-lytic properties or with the capacity to induce tissues to produce bone lysis would seem a reasonable etiologic explanation for the disease¹⁸. On account of the absence of tumor, and on account of numerous differences in clinical behavior myelomatosis was excluded, although the histologic structure of some of the material was very similar to that described by Berkheiser as multiple myelomas in children¹⁹. Furthermore, the infectious theory of the origin of multiple myelomas is as fitting as any. If the infectious hypothesis is true, we may expect to find cases without diabetes insipidus (absence of hypothalamic involvement?) and with other symptoms depending on involvement of structures not materially affected in our case. Clinically and pathologically considered, destruction of membranous bones was the paramount disease process in our case, exophthalmos a mechanical effect, and diabetes insipidus probably a result of associated involvement of the tuber cinereum.

SUMMARY

In a case of defects in membranous bones, exophthalmos and diabetes insipidus studied clinically and pathologically, dyspituitarism as a cause for the syndrome was excluded by the absence of material pathologic changes in the hypophysis. Pathologic support was given to current work on experimental polyuria which puts the lesion of diabetes insipidus in the hypothalamus. From clinical behavior and from pathologic findings an infectious etiology seemed more likely than a primary metabolic (endocrinologic) disturbance.

630 Bankers Reserve Life Building

18 It was suggested that the lung condition by causing tissue asphyxia might have induced calcium absorption and have thus played a part in the bone destruction. One of us (A. D. D.) has seen three cases at necropsy with fully as marked lung findings of a similar character in which there were no skeletal changes.

19 Berkheiser, E. J. Multiple Myelomas in Children, *Arch Surg* 8 853-881 (May) 1924.

LIPEMIA AND THE RETICULO-ENDOTHELIAL APPARATUS ~

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The old blood letters were well aware that under certain pathologic conditions, notably severe diabetes mellitus, the blood may contain an excess of fat so great that, on being left to stand, a thick, creamy layer separates at the top, and that at necropsy creamy masses are found above the clots in the cavities of the heart and in the large vessels throughout the body. It was thought that the presence of such large amounts of fat in the blood stream must of necessity mechanically impede the circulation and perhaps give rise to fat emboli. Best known and most persistent of these hypotheses was the one that attributed diabetic coma to fat embolism of the brain. The untenability of this view was shown by the fact that diabetic coma often occurs in the absence of any considerable lipemia and vice versa. Though the quantity of fat in the blood may be enormous, even surpassing 25 per cent by weight, fat emboli do not occur because of the exceedingly fine state of dispersion of the emulsified fat, in contradistinction to the large drops that enter the circulation following trauma to the bone marrow. Any anatomic findings encountered in a case of lipemia, therefore, are not to be attributed to fat embolism. Nor is there any evidence that the large amount of fat so alters the viscosity or other physical properties of the blood as to increase mechanically the work of the heart.

The first to note any morphologic changes in the organs due to lipemia, so far as we can ascertain, was the Scotch clinician Coats¹. Describing the postmortem findings in two diabetic patients who died in coma with lipemia, he wrote "The spleen had an extraordinary pale color suggestive of salmon roe," and, "The appearance of the spleen in the first case was very peculiar and was such as I have never before observed myself or seen described. On microscopic examination it was seen that there were large cells abundantly present strikingly demarcated by being filled with fine granules, some of which were fatty. The splenic tissues was in great part made up of these large cells."

* From the medical division of the Montefiore Hospital

1 Coats, Joseph. On Two Cases of Diabetes Associated with Lipemia, Glasgow M J 32 95, 1889

This observation of Coats attracted no attention and the condition remained unknown till the article of W H Schultze² in 1912. Schultze described a case of diabetic lipemia in which the splenic pulp was almost completely replaced by large cells with foamy cytoplasm. In fresh, unstained sections these cells appeared filled with shiny droplets, while in alcohol preparations they appeared vacuolated, owing to the presence in the cell body of a lipid substance. This lipid was isotropic, not colored by sudan or osmic acid but intensely stained by the Smith-Dietrich method and by Weigert's medullary sheath stain. He therefore considered the substance, following Kawamura's classification, to be "a lipid in the narrower sense," i e, not containing any considerable amount of either glyceryl or cholesterol esters. Schultze believed the large cells to be derived from the reticular elements and not from the sinus endothelium, which was intact though compressed. The condition was entirely confined to the spleen, no other organs containing the large cells. Following the article of Schultze, instances of lipid cell infiltration of the spleen in diabetes were published by Lutz³ (two cases), Marchand,⁴ Williams and Dresbach,⁵ Eppinger⁶ and Fahr and Stamm,⁷ some of which present significant deviations from the findings of Schultze.⁸ The condition appears to have been very rare, and probably will become even rarer with the introduction of insulin, under the influence of which diabetic lipemia rapidly disappears. In this paper we desire to consider the interpretation of the lesion in the spleen and its relation to certain other morphologic changes likewise attributable to disturbances in lipid metabolism. Our attention was drawn to the condition by the following case, studied in the Montefiore Hospital.

REPORT OF CASE

Milie G, aged 6, was admitted to the medical division suffering from diabetes mellitus. Her diabetes was of the extreme severity seen in early childhood. As it was before the days of insulin, it was impossible to control completely her sugar excretion, which ran as high as 177 gm per day though she weighed only 38 pounds (17.2 kg). Ketonuria also was usually present, as much as 3.8 gm of acetone was excreted daily. It was noted that "the color

2. Schultze. Ueber grosszellige Hyperplasie der Milz bei Lipidaemie, *Verhandl d deutsch path Gesellsch* **15** 47, 1912.

3. Lutz. Ueber grosszellige Hyperplasie der Milzpulpa bei diabetischer Lipaemie, *Beitr z path Anat u z allg Path* **58** 273, 1914.

4. Marchand. *Munchen med Wchnschr* **62** 19, 1915.

5. Williams, J R, and Dresbach, M. A Fatal Case of Diabetes Mellitus Associated with Large Cell Hyperplasia. *Am J M Sc* **153** 65 (Jan) 1917.

6. Eppinger. *Die Hepato-lienalen Erkrankungen*, Berlin, 1920, p 513.

7. Fahr, T, and Stamm, C. Zur Frage der sogenannten Lipoidzellenhyperplasie, *Klin Wchnschr* **3** 1206 (July 1) 1924.

8. After the completion of this paper a new clear-cut case, the second in the American literature was reported by Smith, Margaret G. Hyperplasia of Lipoid Holding Cells in Diabetes with Lipemia, *Bull Johns Hopkins Hosp* **36** 203, 1925. Her findings are in close accord with those of Schultze.

of the skin was that of a low grade jaundice' The edge of the liver was palpable on deep inspiration, but the spleen was not felt After a stay of seventeen months in the hospital, the child died of diabetic coma, Oct 21, 1920

The necropsy⁹ was performed by Dr David Secof The significant findings were briefly as follows

On the palms, backs of the elbows, soles of the feet and, to a less extent, on the reverse surfaces of these parts was a discrete and grouped papular eruption the individual papules varying in size from 1 to 3 mm The older lesions were a bright yellow, the color characteristic of the eruption xanthomatosis diabeticorum

When the heart was opened, the existence of lipemia was noted, a greasy, yellowish white layer of fat separated from the blood

In the endocardium there were numerous slightly elevated, bright yellow patches, each one several millimeters in diameter On the aortic cusp of the mitral valve was a soft, bright yellow plaque involving the greater portion

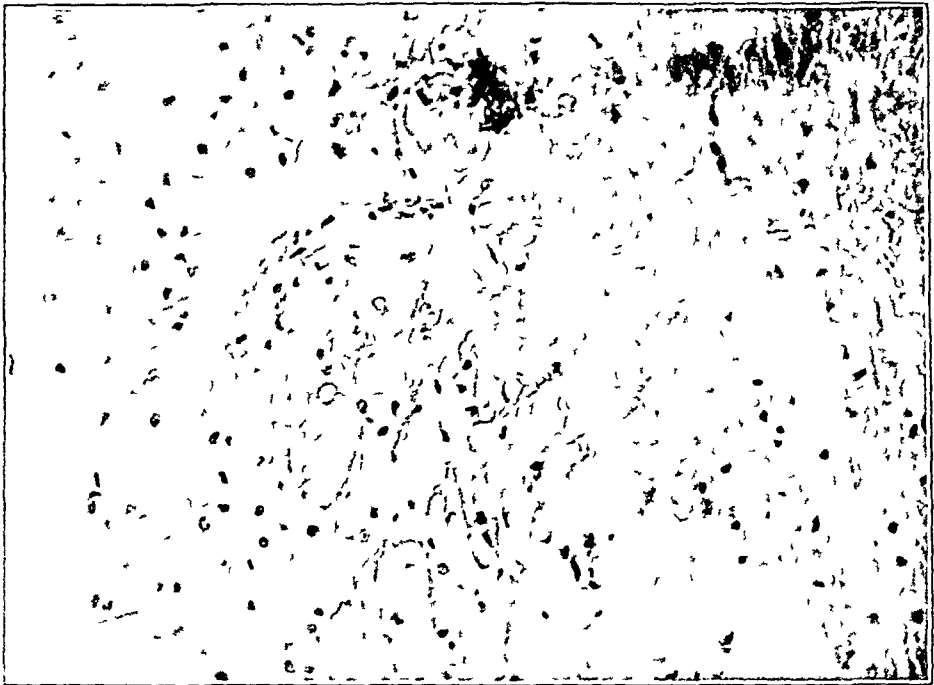


Fig 1—Section of aorta stained with hematoxylin and eosin, high dry power, showing the large lipoid cells in the intima

of this leaflet In the intima of the arch of the aorta, and more prominently in its abdominal portion, were scattered very bright yellow, soft, opaque nodules Microscopically, these nodules were seen to be made up of closely packed polyhedral or round cells (Fig 1) Most of these were very large, but there were small ones and various intermediary sizes Each of these cells had a single small nucleus, often eccentric or pressed to the periphery, imparting a signet ring appearance to the cell The cell body appeared transparent in the paraffin sections, and under the high power was seen to have a "foamy" structure In unstained sections the cytoplasm was seen to be filled with shiny droplets, these stained intensely reddish yellow with sudan By the method of Smith and Dietrich, many of the cells stained steel blue but not very

9 After being for some months in formaldehyd the rather large tissue blocks were unfortunately put in alcohol This apparently did not interfere with the lipoid reaction for the lipemic blood in all the sections stained intensively with sudan

deeply The polarization microscope showed that many of these cells contained an anisotropic body It was seen that some, at least, of the large cells were derived from the endothelial cells, for by following the endothelium between the nodules, various stages between the normal flat cell and the large elements filled with lipoid were to be made out A few mononuclear wandering cells also were present in the intima, whether these cells were likewise concerned in the genesis of the large lipoid cells is difficult to say In a few areas, the outline of the large cells was indistinct and the nuclei could not be seen, their lipoid content coalescing to form larger accumulations The elastic tissue

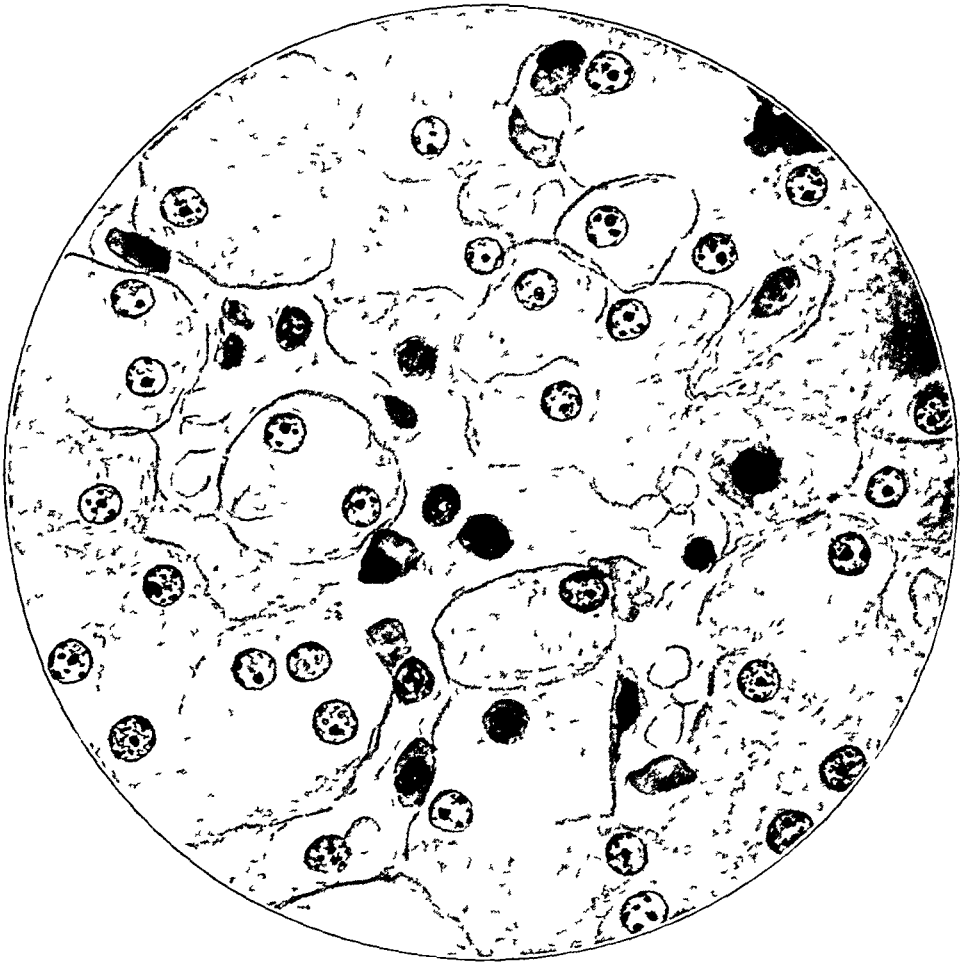


Fig 2—Section of liver stained with hematoxylin and sudan, showing the Kupffer cells loaded with lipoid, oil immersion

of the media was unchanged The blood in the vasa vasorum of the adventitia and outer media stained intensely with sudan and by the method of Smith and Dietrich

The liver weighed 850 gm and was fatty on section Microscopically (Fig 2) the liver cells were seen to contain a small amount of finely divided fat The Kupffer cells were very much enlarged In the paraffin sections the cell body stained but faintly, appearing pale, but not presenting the "foamy" appearance of the large cells in the spleen On the other hand, in frozen sections stained with sudan it was colored orange red, and blue black in the Smith-Dietrich preparations Some of the cells contained an anisotropic body The blood in the capillaries took the sudan and Smith-Dietrich stains very markedly, so that in the latter preparations the capillaries appeared as though filled with a black injection mass (Fig 3)

The capsule of the spleen was thin, and it was of average consistency. A section of the spleen presented a firm, dull grayish surface. The trabeculae were perhaps slightly more prominent than the average. The malpighian bodies were not conspicuous. Most striking was the great increase in gray pulp. Near the spleen there was a filbert sized accessory spleen, similar in appearance on section.

In the paraffin sections, it was seen that the malpighian bodies were very small and widely separated. The pulp was almost completely replaced by large, transparent cells, round or polyhedral in shape (Fig 4). The nucleus was small, stained deeply and was often eccentric. A few of the cells had two nuclei, never more. Under the high power it was seen that the cell body had a foamy appearance, due to the presence of minute circular spaces bounded by a pink staining wall. In some of the cells, these minute spaces had coalesced so as to form large circular vacuoles, which occasionally

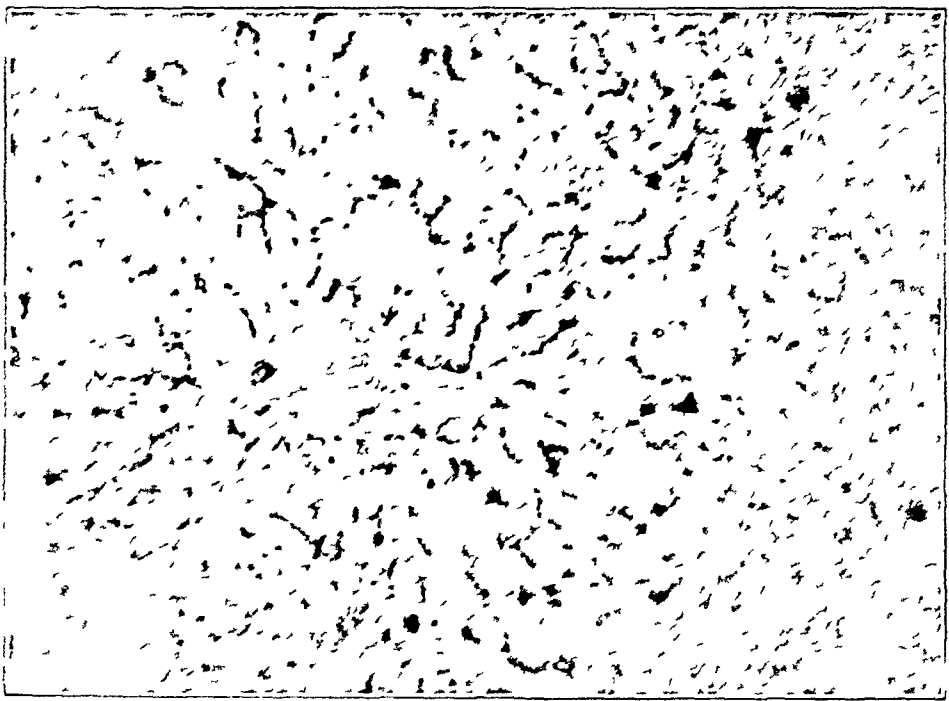


Fig 3—Section of liver stained by method of Smith and Dietrich. The lipid in the capillaries is stained intensely black.

occupied almost the entire cell body. The vacuoles were empty in the paraffin preparations, their contents having been dissolved out in the preparation of the section. In unstained preparations the cells appeared filled with shiny, dark granules. In the sudan sections the blood in the vessels was stained orange red, and around the vessels collections of rather small cells which also stained with sudan were often to be seen. The large cells that had replaced the pulp did not stain with sudan. In the Smith-Dietrich preparations a similar result was obtained, the only positive staining obtained was that of the blood in the vessels and a small number of cells around the vessels. The large cells did not give a lipid reaction at all, or merely very faintly. Only a few sinuses could be made out, and these were compressed. Their lining endothelium seemed intact. The large cells were apparently derived from the reticulum cells, whether or not the sinus endothelium also gave rise to these cells could not be said from our sections. There was considerable hyalin-fatty change of the intima of the smaller arterioles, giving a positive sudan stain.

The lymph glands were not enlarged. No lipid cells were seen in the mesenteric and bronchial glands that were removed.

The cells of the cortex of the suprarenals contained rather less lipid than usual, and that mostly in the glomerular layer.

The pancreas was of average size, the consistency less than usual. The head and a portion of the body appeared digested. There was an area of old hemorrhage in the center. Several sections showed fairly well preserved tissue but a striking absence of islands. No lipid cells were seen.

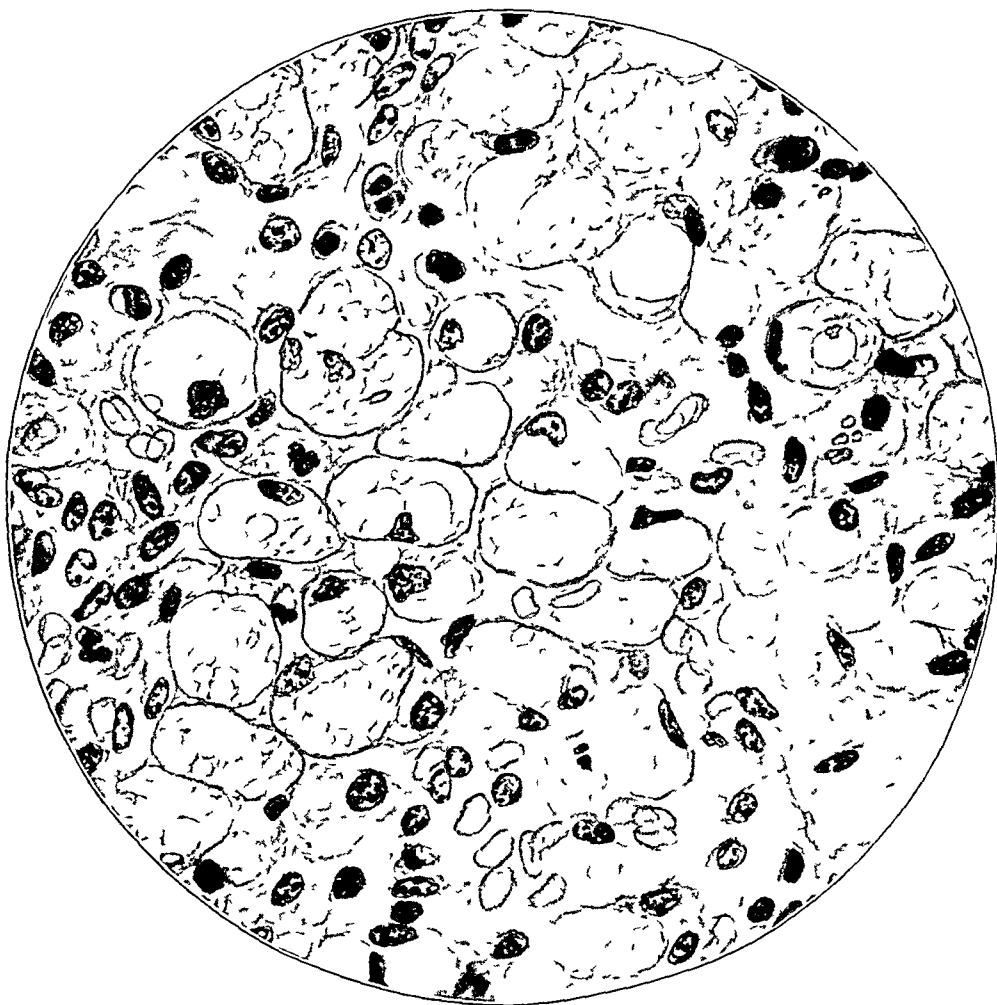


Fig 4—Section of spleen stained with hematoxylin and eosin. The almost complete replacement of the splenic pulp by the large lipid cells (foam cells) is shown, oil immersion.

The glomerular and intertubular capillaries of the kidneys contained large amounts of sudan staining fat. The epithelium of the proximal convoluted tubules contained numerous fine droplets, mostly at the base of the cell, which stained red with sudan. The epithelial cells of the loops of Henle appeared strikingly transparent with well demarcated borders, owing to the presence of large amounts of glycogen in the cytoplasm.

The other organs showed no relevant changes, aside from the large amount of fat in the blood contained in the vessels. Large lipid cells were not noted in any of the other organs. The bone marrow and central nervous system, unfortunately, were not obtained.

The significant features of the foregoing case may be summarized briefly as follows. A child of 6 years with severe diabetes developed acidosis and died in coma. At the necropsy it was seen that a marked lipemia existed. Aside from the changes in the pancreas to which the diabetes was due, there were found the following lesions which we desire to consider in their relations to the lipemia and to one another:

- 1 Extensive infiltration of the splenic pulp by large cells containing lipoid
- 2 Hypertrophy of the Kupffer cells, which contain much lipoid
- 3 Yellow patches in the intima of the aorta and the endocardium, composed of lipoid cells
- 4 Xanthomas of the skin
- 5 A yellowish discoloration of the skin, the so-called xanthosis

THE LIPOID CELLS IN THE SPLEEN

That the spleen is concerned in lipoid metabolism is not immediately obvious, for in the normal spleen neither neutral fat nor lipoid can be demonstrated by histochemical reactions. But several observers (Soper,¹⁰ Eppinger and King,¹¹ and Siegmund¹²) have noted that following splenectomy in dogs and rabbits there is a rise in the neutral fat, cholesterol and phosphatid content of the blood. In man the results reported are discordant, some authors describing a similar rise in the blood lipoids following splenectomy, while others have not observed this.¹³ It would seem that splenectomy is not suited to give us information as to the part played by the reticulo-endothelial cells of the pulp in lipoid metabolism, for these form only a portion of the entire reticulo-endothelial apparatus, and the other parts apparently quickly compensate for them in lipoid metabolism much as they do in the breaking down of hemoglobin.

Of much greater significance are the results obtained by lipoid feeding. In his well known experiments, Anitschkow¹⁴ fed rabbits cholesterol dissolved in sunflower oil over long periods of time. In these animals, in addition to similar changes in the aorta, bone marrow and liver, he

10 Soper. Beziehungen der Milz zum Cholesterinstoffwechsel, Beitr z path Anat u z allg Path **60** 232, 1915

11 King, J. H. Studies in the Pathology of the Spleen, Arch Int Med **14** 145 (Aug.) 1914

12 Siegmund. Milzapparat und Blutlipoid, Munchen med Wchnschr **68** 1035, 1921

13 Dubin, H., and Pearce, R. M. A Note on the Blood Fat Before and After Splenectomy, Arch Int Med **18** 426 (Sept.) 1916

14 Anitschkow. Ueber experimentell erzeugte Ablagerungen von anisotropen Lipoidsubstanzen in der Milz und im Knochenmark, Beitr z path Anat u z allg Path **57** 201, 1914

found that the splenic pulp was almost completely replaced by large "foam cells," loaded with sudan staining, anisotropic lipid, mainly cholesterol esters. Comparison of Anitschkow's drawings with our preparations leaves no doubt of the morphologic similarity of the cells in the spleen of his rabbits with those found in human diabetic lipemia. Anitschkow himself considered the cells in the spleen of Schultze's case as identical with those which he had produced experimentally in the rabbit.

We have mentioned above that in Anitschkow's experiments the lipid contained in the large cells of the spleen is identical with that fed the animal and present in excess in the blood. In this respect, the human condition seems to differ somewhat from the experimental. It is known that in diabetic lipemia the neutral fat, cholesterol and phosphatid fractions of the blood lipoids all increase, the greatest rise being in the neutral fat. In accord with this universal increase in the different blood lipoids, in our case the blood in the lumen of the splenic vessels stained orange red with sudan, steel blue by the Smith-Dietrich method, and exhibited double refraction through crossed Nicols. Similar reactions were given by some rather small fat phagocytes in the immediate vicinity of the vessels, but the great majority of the large cells did not stain with sudan, only faintly or not at all in the Smith-Dietrich preparations, and contained no anisotropic body.

In Schultze's case of diabetic lipemia, the large cells likewise did not stain with sudan or exhibit double refraction, but did stain intensively by the Smith-Dietrich method. Identical reactions were obtained in one of Lutz's cases, but in his other case and that of Williams and Dresbach many (though not all) of the large cells contained sudan staining and anisotropic lipid bodies. It is far from clear why in our case the lipid which stained so well while still in the blood no longer gave the same staining reactions after being stored in the large cells of the spleen. One may advance such purely hypothetic explanations as chemical combination with proteins, changes in the physical state of solution, or elaboration into more complex lipid bodies, but there seems to be no convincing evidence for any of them. In this connection it is important to bear in mind that the so-called lipid staining reactions are often poor indicators of which particular lipid substance is present.

THE KUPFFER CELLS

Normally, the Kupffer cells do not contain histologically demonstrable lipid. Roessle¹⁵ has pointed out that it is very common to find the Kupffer cells containing lipid in diabetes, it is not unusual to find

¹⁵ Roessle. Ueber die Leber beim Diabetes, Verhandl. d. deutsch. path. Gesellsch. **11** 334, 1907.

anisotropic cholesterol esters in these cells in the same disease. This, however, is not universally true, we have recently seen a patient with diabetes dying in coma in whom, though there was extreme deposition of glycogen in the liver cells, the Kupffer cells contained no lipid. But in the lipemic case reported above, the Kupffer cells were much enlarged, their cell bodies were filled with lipid which stained both with sudan and by the method of Smith and Dietrich, and some exhibited double refraction thus differing essentially from the large cells in the spleen. The liver cells themselves contained but little fat.

CHANGES IN THE AORTA AND ENDOCARDIUM

It was mentioned above that the intimal patches are composed of large cells morphologically like those in the spleen but staining deeply with sudan and containing an anisotropic body. It was also seen that some, at least, of these cells are derived from the endothelium, whether other cells also are concerned in their origin could not be said. Anitschkow¹⁶ was able to produce almost precisely the same changes by feeding rabbits cholesterol dissolved in sunflower oil. The cells shown in his illustrations are similar to those in our sections and their staining reactions are the same. He was unable to determine the precise derivation of these cells. In human pathology the existence of these cells in the aortic intima is well known in atherosclerosis, in which they represent wandering cells that have resorbed lipid that has been pressed into the interstices of the intima from the blood plasma. Large collections of such "xanthoma" cells have been described in the intima of the central artery of the retina in hemorrhagic glaucoma¹⁷. In one of Lutz's cases of diabetic lipemia, the presence of large lipid cells in the aortic intima is mentioned. The correlation of such "xanthomas" of the aorta with xanthomas of the skin was noted by Pavy¹⁸ as long ago as 1866. A number of cases have been reported¹⁹ in which cardiac murmurs were caused by xanthomas of the valvular endocardium. Benda²⁰ observed extensive xanthomatosis of the pulmonary veins in a case of nephrosis with marked lipoidemia.

16 Anitschkow. Ueber die Atherosklerose der Aorta beim Kaninchen und ueber deren Entstehungsbedingungen, Beitr z path Anat u z allg Path **59** 306, 1914

17 Velhagen. Ueber den Befund lipoidhaltiger Zellen in der Arteria centralis retinae bei einem Falle von haemorrhagischen Glaukom, Beitr z path Anat u z allg Path **57** 38, 1914

18 Pavy. On a Case of Vitiligoidea Plana et Tuberosa, Guy's Hosp Rep **12** 276, 1866

19 Low. Xanthoma Tuberosum Multiplex with Lesions in the Heart and Tendon Sheaths, Brit J Dermat **22** 109, 1910

20 Benda, Henke and Lubarsch. Handbuch der spez path Anatomie und Histologie, Berlin **2** 826, 1924

THE XANTHOMAS OF THE SKIN

Xanthomas of the skin are bright yellow, flat or papular lesions composed of large cells with small nuclei and cytoplasm filled with lipid so that in paraffin sections they have a characteristic clear or foamy appearance. It was shown by Pincus and Pick²¹ that xanthoma cells contain cholesterol esters and therefore are doubly refractive. A more recent investigation by Fahr²² has revealed that there also are instances in which the predominant substance present is neutral fat. Pollitzer and Wile²³ have found that the formation of a xanthomatous lesion starts with a "proliferation of cells in the immediate neighborhood of the papillary and subpapillary blood vessels," which then take up lipid. The cells observed are evidently the type of phagocytic wandering cells found around blood vessels and termed by Marchand adventitial cells.

There are two main varieties of cutaneous xanthoma.

1 The isolated xanthoma of the eyelid which is so common in old people. The pathogenesis of the lesion is obscure. In particular, there is no manifest lipemia. However, it is of interest to note in this connection that there seems to be a tendency for localized deposition of cholesterol to occur in old age. Thus, besides the xanthomas of the eyelid, atheroma of the large vessels and arcus senilis are exceedingly common findings in healthy old people, the anatomic basis of which consists in the deposition of cholesterol. The arcus senilis has been reproduced experimentally in the rabbit by cholesterol feeding,²⁴ it has also been noted in youthful subjects with hypercholesterinemia²⁵. The reason why the xanthomas occur on the eyelid is unknown, Lubarsch²⁶ believes that lymph stasis is an important factor in determining the location of xanthomatous lesions and this may play a part in determining the eyelids as the site of predilection.

2 Xanthoma multiplex. These lesions are readily recognized by their bright yellow and their predilection for the extensor surfaces of the elbows and knees. They are almost always secondary to a lipoidemia. The most common conditions in which lipoidemia occurs are diabetes, obstructive jaundice and, much more rarely, nephrosis, and these are the

21 Pincus and Pick. Zur Struktur und Genese der symptomatischen Xanthoma, *Deutsch med Wchnschr* **34** 1426, 1908.

22 Fahr. Zur Frage des Xanthoms, *Centralbl f allg Path u path Anat* **30** 609, 1920.

23 Pollitzer and Wile. Xanthoma Tuberosum Multiplex, *J Cutan Dis* **30** 235, 1912.

24 Verse. Ueber einige Organveraenderungen bei der experimentellen Lipo-cholesterinaemie, *Verhandl d deutsch path Gesellsch f Chir* **19** 163, 1923.

25 Benda, discussion of Verse (Footnote 24, page 167).

26 Lubarsch. Generalisierte Xanthomatose bei Diabetes, *Deutsch med Wchnschr* **44** 484, 1918.

diseases in which multiple xanthomas are found. Cases of marked lipoidemia of unknown origin ("idiopathic") with multiple xanthomas have been described.²⁷ A few instances of multiple xanthomas without hypercholesterinemia have been reported,²⁸ it seems quite possible that in these cases the neutral fat or phosphatid content of the blood may have been increased, and these may have been the substances present in the xanthomas. We have recently been able to follow the disappearance of extensive xanthomatosis in a young diabetic with lipemia as the blood lipoids diminished under insulin treatment, proving beyond cavil the dependence of the xanthomas on the excess of lipid in the blood.

THE YELLOWNESS OF THE SKIN

Von Noorden was the first to point out that many diabetic patients have a yellowish discoloration of the skin of the palms, soles and around the mouth which he termed xanthosis diabetico-rum. The yellowish discoloration may become universal and is rarely very intense. It has been shown by Hess and Myers,²⁹ Burger and Reinhart³⁰ and Salomon³¹ that this discoloration is due to carotin and xanthophyll, two fat soluble pigments (lipochromes) which are present in green vegetables, butter, etc. It has been found further that the yellowish pigmentation of the skin is secondary to a similar coloration of the blood plasma that Salomon terms xanthemia. Hess and Myers have shown that this pigmentation can be produced in healthy children by feeding them large amounts of carrots and other vegetables containing lipochromes, Salomon has done this also in adults. A similar pigmentation may occur in infants fed with cod liver oil. Yellowish discoloration of the palms in acute febrile conditions has been termed by the French "la signe palmaire."

An increase in the blood lipochrome, the necessary condition for xanthosis, may or may not accompany an increase in the blood lipoids (neutral fat, cholesterol, phosphatids), the necessary condition for xanthoma multiplex. In the little girl reported above there were both

27 Burns, F. S. A Contribution to the Study of the Etiology of Xanthoma Multiplex, *Arch. Dermat. & Syph.* **2** 415 (Oct.) 1920, also, Fordyce in discussion, p. 428. Arning and Lippmann. Essentielle Cholesterinaemie mit Xanthombildung, *Ztschr. f. klin. Med.* **89** 107, 1920. An instance of this rare "idiopathic" lipoidemia with very extensive xanthomatosis was recently demonstrated at the Mount Sinai Hospital by Dr. A. A. Epstein.

28 Rosenbloom, Jacob. The Cholesterol and Cholesterol-Ester Content of the Blood in Xanthoma Tuberosum Multiplex, *Arch. Int. Med.* **12** 395 (Oct.) 1913.

29 Hess, A. F., and Myers, V. C. Carotinemia. A New Clinical Picture, *J. A. M. A.* **73** 1743 (Dec. 6) 1919.

30 Burger, M., and Reinhart, A. Ueber die Genese der Xanthosis Diabetica, *Deutsch. med. Wchnschr.* **45** 430 (April 17) 1919.

31 Salomon. Ueber Xanthose der Haut, *Wien klin. Wchnschr.* **32** 495, 1919.

xanthoma and xanthosis, but in another case of marked diabetic lipemia recently observed the great lipemia was not accompanied by an increase in lipochrome and the skin was unusually white throughout. Evidently the diet did not contain an excess of vegetable containing lipochrome.

From the foregoing considerations, it is seen that each of the first four findings is secondary to a great increase in blood lipoids. The last mentioned finding, the xanthosis, differs from the others, however, in two essential respects. First, it is due to the accumulation in the blood of specific fat-soluble pigments, lipochromes, and second, instead of being stored within a definite system of cells, as we shall immediately see the lipoids in general are, lipochrome seems to enter almost any variety of cell in which it can dissolve in the cell lipoids.

Consideration of the cells involved in the other four lesions—the reticulum cells of the spleen, the Kupffer cells of the liver, the endothelium and possibly wandering cells in the vascular intima, and the adventitial cells of the small cutaneous vessels—reveals that they all belong to the widely distributed system of cells termed by Aschoff and Landau the reticulo-endothelial metabolic apparatus. The close functional interrelationship of these ubiquitous cells is shown by the fact that after injection into the blood stream of certain dyes (pyrrol blue, trypan red, etc.), they are stored in the reticulo-endothelial system. In cholesterol feeding experiments, the cholesterol is stored in the cells of this system. Similarly, Eppinger³² has found that in normal animals injected iron is stored in the reticulo-endothelial cells. There is much evidence that the formation of bilirubin from hemoglobin occurs within these same cells. Included in the reticulo-endothelial system are three main classes of cells, genetically closely interrelated.

- 1 The reticulum cells of the hematopoietic (spleen, bone marrow and lymph glands) and certain other organs, notably the suprarenals

- 2 Endothelial cells (including the Kupffer cells of the liver capillaries)

- 3 The great group of phagocytic cells in the connective tissues, among which are Ranvier's clasmotocytes of the areolar tissue and Marchand's adventitial cells around the blood vessels

Cells of this system may enter the blood stream, where they are present as monocytic elements (endothelial leukocytes of Mallory)

In our case we have seen that the reticulum cells of the spleen, the Kupffer cells of the liver, the endothelial cells of the aorta and the adventitial cells of the cutaneous vessels have been filled with lipid. Similar lipid storage has been described in cases of lipemia in practically all parts of the reticulo-endothelial system. Thus, Marchand

32 Eppinger Die Hepato-Lienalen Erkrankungen, Berlin, 1920, p. 67

found involvement of the reticulum cells of the bone marrow, Williams and Dresbach and Fahl and Stamm of those of the lymph glands, and there have been described in cases of lipemia due to different causes "xanthomas" (i e, lipid storage) in the most varied portions of the body (pachymeninges, bile ducts, tendon sheaths, periosteum and various viscera), always apparently originating in the reticulo-endothelial cells. In a case of diabetic lipemia, Kawamura³³ demonstrated the presence of cholesterol esters in the monocytic cells of the blood, which we have mentioned above as partially derived from the reticulo-endothelial system.

From the foregoing case and the facts gathered from the literature, it is seen that when a great excess of lipid accumulates in the blood, which occurs most usually in diabetes and chronic obstructive jaundice, it is stored within the reticulo-endothelial system. This may result in varied anatomic pictures (which have hitherto not been sufficiently correlated with one another), for the portion of the system in which the lipid is deposited in an individual case varies greatly without our knowing the reason why. Thus, the reticulum cells of the spleen may be the only portion involved, the lymph glands may also participate, or there may be only xanthomas of the skin, etc. The same holds true of other varieties of reticulo-endothelial storage, for instance, of dyes or of iron. That different staining reactions are given by the stored fat and lipid in various portions of the reticulo-endothelial system was pointed out above.

The histologic appearance of the spleen in cases of lipid cell hyperplasia is very much like that observed in Gaucher's splenomegaly, in which the splenic pulp is also replaced by large, clear cells, so that it is not surprising that Schultze, in describing the condition, voiced the opinion that Gaucher's disease is also a disturbance of lipid metabolism. This view has been supported by Wahl and Richardson³⁴ on the basis of material studied in conjunction with Knox and Schmeisser, but Mandlebaum and Downey³⁵ have shown clearly that their cases were not Gaucher's disease. However, it has been pointed out by Mandlebaum³⁶ and by Pick³⁷ that the substance contained in the large cells

33 Kawamura. *Die Cholesterinverfettung*, Jena, 1911, p. 88.

34 Wahl, H. R., and Richardson, M. L. A Study of the Lipin Content of a Case of Gaucher's Disease in an Infant, *Arch Int Med* **17** 238 (Feb) 1916.

35 Mandlebaum, F. S., and Downey, H. The Cases of Gaucher's Disease Reported by Drs. Knox, Wahl and Schmeisser, *Bull Johns Hopkins Hosp* **27** 109 (April) 1916.

36 Mandlebaum, F. S. Two Cases of Gaucher's Disease in Adults, *Am J M Sc* **157** 366 (March) 1919.

37 Pick, Ludwig. Zur pathologischen Anatomie des Morbus Gaucher, *Med Klin* **18** 1408, 1922, Ueber den Morbus Gaucher, seine Klin., pathologische Anatomie und histo-pathologische Umgrenzung, *ibid* **20** 1403 (Oct 12) 1924.

of Gaucher's disease (the so-called Gaucher substance) never gives lipid reactions, and that the cells differ somewhat both morphologically and in distribution from those found in cases of lipemia. Mandlebaum states that the demonstration of histochemical lipid reactions in the large cells in any case immediately rules out the diagnosis of Gaucher's disease. In his recent masterly study of Gaucher's splenomegaly, Pick points out that in this disease the endothelial elements of the reticulo-endothelial system are not involved, though we have seen that they are in cases of lipemia. Considering these well established facts, it would seem that the two conditions have nothing more in common than a superficial morphologic similarity of the large, clear cells making up the lesions. Pick himself considers Gaucher's disease as a "congenital, familial, constitutionally caused anomaly of metabolism akin to alkaptonuria or cystinuria."

But in recent investigations Epstein³⁸ and Lieb³⁹ have shown that the "Gaucher substance" stored in the large cells characteristic of the disease contains a considerable proportion of kersasin, one of the cerebrosids, these are complex lipoids and are usually found in association with other lipid bodies. They also found that the Gaucher cells contain large amounts of alcohol-soluble, but ether-insoluble phosphatids. So it would seem that, despite the negative histochemical findings, *Gaucher's disease is an anomaly of lipid metabolism in which complex lipoids fail of complete disintegration and elimination and are stored in the histiocytic elements of the reticulo-endothelial system*.

Just as there are various metabolic anomalies in which the intermediary metabolism of protein stops at a certain point (e g, alkaptonuria, in which the organism is unable to break down homogentisic acid), so there are apparently different disturbances of lipid metabolism. These may be secondary and acquired, as in diabetes, or primary and constitutional, as in Gaucher's disease. Another primary disturbance of lipid metabolism has recently been described by Pick³⁷ under the name of "lipoid cell splenohepatomegaly," by which he understands a "congenital, familial, constitutionally caused anomaly of lipid, especially phosphatid, metabolism." In all four reported cases, this has led to death within the first two years of life, the striking anatomic finding being the presence of large lipid cells in various organs. In these cases as well as in diabetic lipemia and Gaucher's disease, the large cells constituting the lesions are merely evidence of storage of different lipoids present as a result of a metabolic derangement.

38 Epstein, E. Beitrag zur Chemie der Gaucherschen Krankheit, Biochem Ztschr **145** 398, 1924

39 Lieb, Hans. Cerebrosidspeicherung bei Splenomegalie, Typus Gaucher, Ztschr f physiol Chem **140** 305, 1924

SUMMARY

1 In experimental lipid feeding, the lipoids are stored in the reticulo-endothelial cells. This lipid storage manifests itself by the occurrence in various organs of lesions that are made up of large, clear cells ("foam cells") filled with lipid.

2 Lesions identical in structure and distribution with those of experimental lipemia are found in diseases in which the lipid content of the blood is increased, such as diabetes, obstructive jaundice and nephrosis.

3 These lesions are also made up of large foam cells containing lipid. The foam cells are derived from the reticulo-endothelial cells, and collections of them may occur in various parts of the body (so-called visceral xanthomas). In the past the various lesions thus produced have not been sufficiently correlated with one another. In the skin they appear in the form of multiple xanthomas.

4 A case of diabetic lipemia is described in which the lipid storage is manifested by

(a) Almost complete replacement of the splenic pulp by large foam cells

(b) Marked deposition of lipid in the enlarged Kupffer cells

(c) Yellow patches in the intima of the aorta and the endocardium composed of foam cells

(d) Xanthomas of the skin

5 The yellowish discoloration of the palms and soles (xanthosis) seen in diabetes is not due to lipemia *per se* but is secondary to an increase in the lipochrome (carotin and xanthophyll) content of the blood. Increase in blood lipochrome and lipemia may or may not be associated.

6 In both manifest lipemia and Gaucher's disease the lesions are composed of cells of the reticulo-endothelial apparatus in which lipoids are stored. But in Gaucher's disease the disturbance of lipid metabolism is primary and apparently constitutional, it is morphologically manifested by the storage in the histiocytes of cerebrosids and other complex lipoids which do not give the lipid staining reactions. In the lipemic cases, the derangement of lipid metabolism is secondary (diabetes, icterus, etc.) and the lipoids found in the reticulo-endothelial cells are those which circulate in excess in the blood (neutral fat, cholesterol and its esters, and phosphatids).

RENAL INJURIES BY AMINO-ACIDS *

L H NEWBURGH, M D

AND

PHIL L MARSH, M D

ANN ARBOR, MICH

It has been shown by Newburgh and his collaborators ¹ (1919, 1923) that high protein diets produce renal injury in rabbits, and by Polvogt, McCollum and Simmonds ² (1923) that similar diets have a harmful effect on the kidneys of rats. In explanation of this fact, a number of possibilities present themselves for consideration, among which are (1) poisonous products arising from the action of bacteria on the food in the intestine, (2) the absorption of foreign protein forced into the blood by the excess in the intestine, (3) the excretion of an acid urine from diets high in certain proteins, (4) extra work required of the kidney by the increased nitrogen metabolism, and (5) lack of vitamins. No evidence in favor of any of these explanations was obtained.

In a previous article, it was suggested that the amino-acids themselves might be the source of the renal injury. That both the absorption and the excretion of amino-acids by the kidney is increased by the ingestion of large amounts of protein has been clearly demonstrated by Levene and Van Slyke ³ (1912) and by Folin and Berglund ⁴ (1922). This article deals with the effects on the kidney of the administration of some of the amino-acids.

METHODS

In this study, the amino-acids entered the body by the intravenous route. In a few of the earlier experiments with rabbits the amino-acids were given in relatively small amounts of fluid. The development of certain general symptoms without evidence of renal injury in some of these animals suggested to us that they were caused by the hypertonicity of the injected solution. We accordingly increased the dilution so that in every case, when it is not otherwise stated, 20 c c of the solution contained 1 gm or less of the amino-acid. Physiologic sodium

* From the Department of Internal Medicine, University of Michigan Medical School.

1 Newburgh, L H. Production of Bright's Disease by Feeding High Protein Diets, *Arch Int Med* **24** 359 (Oct) 1919. Newburgh, L H, and Clarkson, S. Renal Injury Produced in Rabbits by Diets Containing Meat, *Arch Int Med* **32** 850 (Dec) 1923.

2 Polvogt, L M, McCollum, E V, and Simmonds, N. *Bull Johns Hopkins Hosp* **34** 168 (May) 1923.

3 Levene, P A, and Van Slyke, D D. *J Biol Chem* **12** 301, 1912.

4 Folin, O, and Berglund, H. *J Biol Chem* **51** 413 (April) 1922.

chlorid solution was used as the vehicle, and precautions were taken that none of the solutions were acid or more than slightly alkaline, except in the case of Rabbit 1 and in certain experiments with glutamic acid, which are discussed in their proper place

The rabbits used were selected healthy animals that had not been previously employed for any other laboratory purpose. The solution was injected into the ear vein at body temperature without an anesthetic. Because of the prevalence of chronic kidney disease in adult dogs, only puppies recently weaned were used. A very few were obtained from animal dealers, but most of them were undernourished or the subjects of infection and were therefore unsuitable for our purpose. Nearly all the dogs used in these experiments were secured as follows. Pregnant bitches or mothers with recently born litters were obtained from neighboring farmers. Each family was housed in an individual kennel out of doors. A few days after the puppies were weaned they were used one at a time, the others remaining with the mother. During the experimental period, each dog was kept in a clean metabolism cage or a pen reserved for that purpose. Strict isolation from other dogs that constituted a possible source of infection was maintained. These precautions were so successful that the young animals appeared almost without exception to be entirely healthy. Very satisfactory anesthesia was obtained by the subcutaneous injection of morphin sulphate in doses of from one-quarter to three-quarter grain (0.01 to 0.04 gm.), depending on the size of the dog. The vein to be used in the case of dogs was prepared by cutting down on it with the usual surgical precautions. All dogs marked with the same capital letter following the number were litter mates.

In the case of both dogs and rabbits, those showing urinary abnormalities before receiving an amino-acid were permanently rejected and only those whose urine was normal on two successive examinations were used.

The solution was injected slowly with a syringe or allowed to flow in from a buret through a needle, depending on the volume. When the amount of fluid was large, the inflow period was as long as two hours, and when the volume was more than a few cubic centimeters, it was maintained at approximately body temperature. For this purpose the buret was enclosed in a constant temperature jacket.

Following the injection of an amino-acid, the urines were examined daily or oftener for the presence of albumin, casts and red blood cells. We found, as have other workers, that it is not unusual for clear rabbits' urine to become cloudy as the result of boiling and acidulating, and further that the cold acidified specimens would sometimes slowly develop a similar cloud. We therefore concluded that when dealing

with rabbits' urine the ordinary tests for albumin give unreliable evidence of renal disease. While we did not experience this difficulty with dogs' urine, we nevertheless did not consider any urine, either rabbits' or dogs', obtained after the injection of an amino-acid as abnormal which did not show in the centrifugated specimen at least several undoubted casts when examined in the usual way.

We had hoped that careful weighing of the kidneys would give us quantitative information regarding their condition. We found, however, that there was a large variation in the weight of the kidneys of normal animals per kilogram of body weight. We therefore discarded that method of judging the state of the kidneys. Likewise, we found it unsatisfactory to draw conclusions from the gross appearance of the kidneys, since the color variations were great in untreated animals. In a few of the "treated" dogs, the signs of congestion or of cloudy swelling and edema were so striking that there could be no doubt of their presence. Likewise, in the case of several dogs the signs of hemorrhage were so marked that abnormality was certain.

Some of the animals were killed at varying intervals after the administration of the amino-acids and their kidneys immediately placed in fixing solution. The histologic evidence of abnormality was entirely satisfactory.⁵ Most of the kidneys so studied were from dogs and the kidneys of untreated litter mates were used as controls.

Some animals whose urine became abnormal after injection were allowed to recover. This gave some information regarding the duration of the injury. Furthermore, recovery from abnormalities that occurred immediately after the injection gave evidence that they were actually due to the injection.

The nonprotein nitrogen of the blood was determined in some of the rabbits. Abnormally high readings yielded further evidence of renal damage following the injection of some of the amino-acids.

EXPERIMENTAL DATA

The experiments with each amino-acid are brought together in one table. Every animal that received an injection is included. Because of intercurrent disease, conclusions could not be drawn from a few of the animals, and these experiments are grouped in a special section of the appropriate table.

Alanin.—When alanin is dissolved in salt solution, a reaction neutral to phenol red results. It is so easily soluble that two of the rabbits received large doses of the alanin in relatively concentrated form.

⁵ The tissues were all fixed in a 4 per cent solution of formaldehyd. Thin sections were cut from paraffin blocks and were then stained with hematoxylin and eosin.

instead of at the rate of 1 gm to 20 c c of diluent, which we later adopted as our standard in order to obviate certain general disturbances that we attributed to the hypertonicity of the injected solution. In spite of this, none of the animals showed either renal or general disturbances following the intravenous injection of 2 gm per kilogram.

Leucin—In order to get leucin into solution, we found it necessary to keep it hot on the water bath for some minutes in a volume of at least 25 c c of the salt solution for each gram of the substance and to add 1.15 c c standard sodium hydroxid⁶ per gram of leucin. The resulting solution was very slightly alkaline in phenol red. None of the three animals that received the leucin gave any evidence of injury, even though Rabbit 30 was given 2.7 gm per kilogram.

The negative result with the dog is of importance as a control for some of the later experiments with amino-acids that produced renal damage, since the outcome of this experiment demonstrates that the intravenous injection of a large volume of fluid does not injure the kidneys. This dog received 250 c c of fluid at the rate of 82 c c per kilogram.

Glycin—This is easily soluble in salt solution and the reaction is neutral. As was the case with alanin, we gave some of the rabbits in this series solutions that were too concentrated. We attributed the sudden death of Rabbit 4 to this cause, and surmised that the prolonged disturbance exhibited by Rabbits 3 and 5 might have a similar origin. At least, none of the animals that were injected with solutions of standard dilution showed any general or renal disturbances. The reasons for not accepting three of the experiments of the series are too evident to require special comment.

We obtained no evidence that glycine, even in doses as large as 3.4 gm per kilogram, injured the kidney.

Phenylalanin—The solution of phenylalanin was neutralized with 4 c c of standard sodium hydroxid per gram of amino-acid. The slightly abnormal urine obtained from Rabbit 29 following the injection of 1.6 gm per kilogram was probably not caused by the phenylalanin, since a much larger dose given to Rabbit 41 did not cause the urine to become abnormal. The failure to injure the kidneys of Dog 11 with 3.7 gm per kilogram left us with no convincing evidence that renal damage is produced by intravenous injection of phenylalanin.

⁶ The solution of sodium hydroxid used in this and many subsequent experiments was old. It had originally been normal, but was only approximately so when we employed it. We accordingly designate this solution as "standard" rather than "normal." In later experiments, when we speak of "normal sodium hydroxid," we refer to a freshly prepared accurately titrated solution.

TABLE 1—*Alanus*

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 15	2,300	0.09 (0.2) 5 times in 5 days	Four examinations before injection, negative, six specimens during period of injection, negative	No necropsy	
Rabbit 10	?	(5) once in 30 c c of solution	Two specimens before injection, negative, four specimens following injection, negative	No necropsy	
Rabbit 16	?	(5) once in 25 c c of solution	Two specimens before injection, negative, four daily specimens following injection, negative	No necropsy	
Dog 53 K	2,800	2 (5.6) once	Two specimens before injection, negative, two specimens following injection, negative	No histologic abnormalities seen in the kidney	Killed 48 hours after injection

* Figure in parenthesis is total single dose

TABLE 2—*Leucine*

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 30	1,650	2.7 (4.5) once	Two specimens before injection, negative, three specimens following injection, negative	No necropsy	
Rabbit 32	2,250	2.2 (5) once	Two specimens before injection, negative, two specimens following injection, negative	No necropsy	
Dog 59 K	3,050	2 (6.1) once	Two specimens before injection, negative, two specimens following injection, negative	No histologic abnormalities seen in the kidney	250 c c of solution injected, which was at the rate of 82 c c per kilogram

* Figure in parenthesis is total single dose

TABLE 3—*Glycin*

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 4 A	?	(0.5) once, (1) once in 8 c.c. of solution, interval 24 hours	Two specimens before injection, negative, four specimens following injection, negative	No necropsy	
Rabbit 3	1,900	2.2 (4.2) in 17 c.c. of solution once	Urine normal before injection, three daily specimens following injection, negative	No necropsy	Ate little and was lethargic for several days after injection
Rabbit 51	2,450	2 (4.9) once	Urine normal before injection, two daily specimens following injection, negative	No abnormalities seen	Killed 48 hours after injection
Rabbit 38	1,450	3.4 (5) once	Two specimens before injection negative, two daily specimens following injection, negative	No necropsy	
Dog 2 A	7,650	1.3 (10) once	Urine before injection, negative, two following injection, negative	No necropsy	
Dog 53 K	2,500	2 (5)	Two specimens before injection, negative, two daily specimens following injection, negative	Slight cloudy swelling, few cists in medulla	Killed 48 hours after injection
			Experiments Not Acceptable		
Rabbit 9	2,350	0.17 (0.4) 5 times in five days, two weeks later 2.1 (5) once	Daily examination of urine, negative until two days after large injection, when many cists were seen	No necropsy	Large abscess of neck noted coincident with abnormal urine
Rabbit 4	1,600	3.1 (5) in 20 c.c. of solution once	Urine normal before and after injection	No necropsy	Convulsion and death 2 hours after injection
Rabbit 5	1,650	3 (5) in 20 c.c. of solution once	Three specimens before injection, negative, two specimens following injection showed albumin and casts	No necropsy	Ate little and was lethargic after injection, three days after injection, had a convulsion and died

* Figure in parenthesis is total single dose

TABLE 4—*Phenylalanin*

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 29	1,800	0.5 (1) once, two days later 1.6 (3) once	Two specimens before injection, negative, on second and third days after second injection, albumin and a few casts were present, thereafter, urine was negative	No necropsy	
Rabbit 33	1,500	0.66 (1) twice, interval 48 hours	Two specimens before injection negative, two specimens following first injection, negative	No necropsy	Sudden death following second injection
Rabbit 41	1,750	2.8 (5) once	Two specimens before injection, negative, two specimens following injection, negative	No necropsy	
Dog 11	2,700	3.7 (10) once	Two specimens before injection, negative, three specimens following injection, negative	No microscopic abnormalities seen	Killed 3 days after injection

* Figure in parenthesis is total single dose

Glutamic Acid—A number of the experiments in this series could not be accepted because the animals either died at the end of the injection or were ill following it. All of these animals received unneutralized solutions of glutamic acid. Furthermore, the solutions were too concentrated. The injection of dilute solutions made neutral to phenol red with standard sodium hydroxid caused no detectable renal injury. We accordingly concluded that the abnormal urine voided by Rabbit 9 was attributable to the acidity of the solution. The data obtained from Dog 57 K are valuable as control material. The large amount of combined alkali, 48 c c of standard sodium hydroxid, or more than 14 c c per kilogram, used to neutralize the glutamic acid did not damage the kidneys, even though the dog developed manifest tetany toward the end of the experiment.

Arginin—The alkaline solution formed by dissolving arginin in salt solution was neutralized against phenol red with standard hydrochloric acid solution at the rate of 3.5 c c per gram of the amino-acid. The five acceptable rabbit experiments taken together give satisfactory evidence of the injurious effect of arginin on the kidney. But the injury is mild as compared with the effect of some of the amino-acids to be dealt with later, and arginin is apparently incapable of damaging the dog's kidney even when large doses are injected.

Aspartic Acid—The acid solution was neutralized against phenol red with 8 c c of standard sodium hydroxid per gram of the amino-acid. The rabbit injections show that well marked renal injury is produced in them by a single injection of 1 gm per kilogram. As the result of an injection of that size, Rabbit 56 voided urine containing casts for eight days subsequent to it. The evidence obtained from dogs is insufficient to prove that aspartic acid injures their kidneys in the dosage employed.

Here again we obtained evidence that the presence of a very large amount of alkali added to the injection fluid to neutralize the acidity of an amino-acid does not injure the kidneys. In six days Dog 25 D received 55.2 c c of standard sodium hydroxid twice, followed two days later by 83.2 c c of standard sodium hydroxid, a total of 138.4 c c, or 56 c c per kilogram, added to a solution of aspartic acid to make it neutral to phenol red. This animal exhibited no urinary abnormalities.

*Lysin*⁷—The lysin was injected in the form of the dichlorid, but the dosage is calculated as the lysin itself. We would have liked to perform more experiments with it, but were unable to obtain any more of it.

⁷ We are indebted to Professor H. B. Lewis for the lysin dichlorid used in these experiments.

TABLE 5—*Glutamic Acid*

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 11	?	(1) once not neutralized 3.7 (5) once	Two specimens preceding and four following injection were negative	No necropsy	
Rabbit 39	1,350		Two specimens before injection, negative, two specimens following injection, negative	No necropsy	
Dog 57 K	3,300	2 (6.6) once	Two specimens before injection, negative, two daily specimens following injection, negative	No histologic abnormalities seen in the kidney	18 cc of normal sodium hy- droxid were required to neu- tralize the glutamic acid violent tetany occurred at end of injection
Rabbit 12	?	(2) in 10 cc of solu- tion not neutralized	Experiments Not Acceptable	No necropsy	Ill for several days after in- jection
Rabbit 14	?	(3) in 15 cc of solu- tion not neutralized	Urine normal before injection, four daily speci- mens following injection, negative, five specimens before injection, negative	No necropsy	Ill for several days after in- jection
Rabbit 7	?	(7) in 30 cc of solu- tion not neutralized	Three specimens before injection, negative, urine voided fifteen minutes after injection showed albumin and casts	No necropsy	Died in convulsions shortly after injection
Rabbit 9	?	(5) in 30 cc of solu- tion not neutralized	Two specimens before injection, negative, daily after injection, urine contained albumin and casts	No necropsy	Ill following injection
Rabbit 15	?	(5) in 30 cc of solu- tion not neutralized	Two specimens before injection, negative, three specimens following injection, negative	No necropsy	Ill for several days following injection

* Figure in parenthesis is total single dose

TABLE 6—*Asigum*

Animal	Weight, Gm	Dose per Kilogram * Gm	Urine	Histology	Remarks
Rabbit 55	3,150	0.5 (1.6) once	Two specimens before injection, negative, sec- ond day after injection, urine contained much albumin but no casts, thereafter, urine was normal	No necropsy	
Rabbit 58 Rabbit 53	2,650 2,750	1 (2.6) once 1 (2.7) once	Urine normal before and after injection Two specimens before injection, negative, two daily specimens after injection showed albumin and many casts	No necropsy Many old linear scars but no acute changes	Killed 48 hours after injection liver was moderately affected by coccidiosis, lungs pre- sented a number of granular areas which exuded pus on section
Rabbit 60 Rabbit 52	1,950 3,400	1.5 (3) once 2 (6.8) once	Urine was negative before and after injection Two specimens before injection negative two daily specimens following injection showed much albumin and many granular and hyaline casts	No necropsy Many old linear scars causing bands of contraction in cortex	Killed 48 hours after injection
Dog 24 C	1,800	2 (3.6) twice, interval 48 hours, 2.5 (4.6) once interval 48 hours	Two specimens before injection, negative five specimens during injection period, negative	No necropsy	
Dog 62 L	2,500	2 (5) once	Urine was negative before and after injection	No abnormalities	Killed 48 hours after injection
Dog 20	2,050	2 (4.1) twice, interval 48 hours	Urine was negative before and after injection Experiment Not Acceptable Urine was negative before and after injection		Morning after second in- jection, dog had a bloody diarrhea, no further obser- vations made

* Figure in parenthesis is total single dose

TABLE 7—*Aspartic Acid*

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 59	1,550	0.5 (0.92) once	Two specimens before injection, negative, occasional casts on day after injection, thereafter, urine again normal	No necropsy	
Rabbit 56	2,700	1 (2.7) once	Two specimens before injection, negative, for the first five days after injection the urine contained albumin and many hyaline, granular and cellular casts, thereafter, casts were present in decreasing numbers until the ninth day when the urine was again normal	No necropsy	
Rabbit 54	2,500	2 (5) once	Urine normal before injection, three hours after injection, albumin and many casts present, two specimens on day following injection contained many granular, hyaline and cellular casts	Several deep linear scars extending from capsule to medulla, numerous casts	Killed 24 hours after injection
Dog 45 G	5,250	1.8 (9.7) once	Two specimens before injection, negative, one specimen 18 hours after injection, negative	No microscopic abnormalities were seen	Killed 2 days after injection
Dog 17	5,400	2 (10.8) once	Two hours after injection, albumin and a few casts noted, 18 hours after injection, both albumin and a few casts were found, thereafter, casts were not seen	No necropsy	
Dog 25 D	3,450	2 (6.9) twice, interval 48 hours, 3 (10.4) once, interval 48 hours	Urine normal before and after injections	No necropsy	
Rabbit 57	2,250	0.5 (1.1) once	Experiments Not Acceptable Two specimens before injection, negative, day after injection, much albumin and many casts were present		Day after injection, purulent nasal discharge, next morning found dead
Rabbit 59	2,800	2 (5) once	Day following injection, much albumin and many casts		Found dead morning after injection, necropsy revealed bronchopneumonia
Dog 16	4,200	3.0 (12.6) once	Four samples before injection, negative		Found dead morning after injection

* Figure in parenthesis is total single dose

TABLE 8—*Lysin*

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 49	1,800	0.18 (0.33) once, inter- val 24 hours, 0.11 (0.2) once	Before injection, negative, after first injection a few casts were seen, 48 hours after second injection, many casts were found, each of seven examinations during next eleven days showed numerous casts, albuminuria was variable Two specimens before injection were negative, 48 hours after second injection, albumin and a few casts present, albuminuria continued three days, but casts were not seen again	No necropsy	
Rabbit 50	2,000	0.16 (0.33) once, inter- val 24 hours, 0.27 (0.54) once, inter- val 48 hours, 0.2 (0.4) once	Two specimens before injection, negative, morn- ing after injection, urine negative, 48 hours after injection, albumin and casts present Two specimens before injection, negative, urine remained normal after injection	No necropsy	
Dog 46 H	3,550	0.6 (2.2) once	Two specimens before injection, negative, morn- ing after injection, urine negative, 48 hours after injection, albumin and casts present Two specimens before injection, negative, urine remained normal after injection	No histologic abnormalities were seen	Killed 48 hours after injection
Dog 33	2,850	1.35 (3.8) once	Two specimens before injection, negative, three specimens during 48 hours following injec- tion each showed numerous granular and cellular casts	Congestion, edema, vacuolar de- generation of epithelial cells lining straight tubules	Four days after injection was killed
Dog 42 G	3,000	1.3 (4.0) once	Two specimens before injection, negative, three specimens during 48 hours following injec- tion each showed numerous granular and cellular casts	Moderate cloudy swelling and granular degeneration of epithe- lium of convoluted tubules	Killed 48 hours after injection
Rabbit 48	2,050	0.65 (1.3) once	Experiment Not Acceptable Negative before injection		During injection had a convul- sion and died

* Figure in parenthesis is total single dose

TABLE 9—*Histidin*

Animal	Weight, Gm	Dose per kilo, gram,* Gm	Urine	Histology	Remarks
Rabbit 1	1,400	0.10 (0.10) every fourth day, ten injections	Three specimens before injection, negative, 18 hours after first injection, a few casts were seen, albumin did not appear until after third injection, both albumin and casts increased slowly until the albuminuria became very heavy and the casts very numerous, both still marked eight days after last injection. Four specimens before injection, negative, it remained normal throughout the first series of injections until the last day when a few casts were noted, during the second series of injections, casts were present at every examination. Urine specimens before injection, negative, remained negative throughout first series of injections, casts appeared after first injection of second series, gradually increased in number, and were regularly found for four weeks after last injection, thereafter, urine was again negative.	Reference should be made to protocol of Rabbit 1 and 5	Killed 9 days after last injection
Rabbit 6	2,200	0.06 (0.11) every third day, five injections followed by 0.15 (0.31) every third day, six injections	Urine normal before injection, a few casts were found after second injection, they were found repeatedly on subsequent examinations.	Most of the glomeruli showed delicate adhesions between tufts and capsule, cysts were numerous	Two days after last injection blood nonprotein nitrogen was 36 mg per 100 cc of plasma
Rabbit 7	2,000	0.06 (0.10) every third day, five injections followed by 0.15 (0.31) every third day, six injections	Urine normal before injection, a few casts were found after second injection, they were found repeatedly on subsequent examinations.	No necropsy	Two days after last injection blood nonprotein nitrogen was 36 mg per 100 cc of plasma
Rabbit 3	1,500	0.23 (0.31) daily, four injections	Urine normal before injection, neither albumin or casts, day after injection, albumin but no casts were found, next day, both albumin and casts were found.	No necropsy	Twenty four hours after last injection, blood nonprotein nitrogen was 63 mg per 100 cc
Rabbit 2	1,200	0.13 (0.52) once	Urine before injection showed neither albumin or casts, day after injection, albumin but no casts were found, next day, both albumin and casts were found.	A few casts, otherwise not abnormal	Killed 18 hours after injection
Rabbit 1	1,450	0.5 (0.69) in 6 cc of solution (not neutralized) once	Urine normal before injection day after injection, urine contained much albumin and many casts, both were abundantly present for next seven days, when urine again became normal.	No necropsy	After nothing for 18 hours following injection, had loose movements for three days after injection
Dog 55 K	3,100	0.33 (1.0) every other day, five injections	Two specimens before injection, negative, during injection period a few casts were found in three specimens and were absent in three.	The convoluted tubules stand out because of the deeper pink stain of their lining cells, which are swollen and hazy and in places flayed out and granular, a few nuclei are pyknotic	Killed 48 hours after last injection
Dog 1 A	7,050	0.5 (3.1) twice, four day interval	Urine normal before injection, heavy albuminuria and many casts were present day after first injection and continued for eight days after second injection, when urine again became normal.	No necropsy	After nothing for 18 hours following injection, had loose movements for three days after injection
Dog 19 I	3,650	1.38 (5) once	Two specimens before injection, negative, first granular and epithelial casts after injection, urine contained numerous granular and epithelial casts.	The cortical tubules showed moderate cloudy swelling, scattered casts seen	Killed 18 hours after injection
Dog 61 K	2,000	1.21 (3.6) once	Two specimens before injection, negative, first day after injection, urine contained a very few casts and no albumin, the second day casts were more numerous and albumin was present, the third day there were many casts and albumin	No histologic abnormalities seen in kidneys	Killed third day after injection

* Figure in parentheses is total single dose

When lysin dichlorid is dissolved in salt solution, an acid reaction is produced. We used 1.9 c.c. of standard sodium hydroxid per gram of the amino-acid to make the reaction of the solution neutral to phenol red.

The five successive experiments demonstrate by means of urinalysis, as well as by the microscopic examination of the kidney, that lysin is definitely nephrotoxic. We were unable, with the limited supply of lysin, to determine how small a dose will injure the kidney.

Histidin—This was used in the form of the dichlorid, but the dosage was calculated as histidin itself. When the dichlorid was dissolved in salt solution, the resulting acidity required 8 c.c. of our standard solution per gram of the amino-acid to obtain neutrality with phenol red or bromcresol purple. The first experiment with histidin was performed on Rabbit 1. This is the only experiment in which the injection fluid was not neutralized. But the striking evidence of kidney injury in this experiment is not attributable to the acidity of the solution (the equivalent of 8 c.c. of standard alkali) since much larger amounts of acid in the form of glutamic acid were injected without evidence of renal injury. Rabbit 15 received the equivalent of 36 c.c., Rabbit 14, the equivalent of 22 c.c., Rabbit 12, the equivalent of 14.5 c.c. standard acid as glutamic acid without the occurrence of abnormal urines.

The most interesting data in this series were obtained from the comparative study of Rabbit 4, which received the injections of histidin, and Rabbit 5, which was the control. The accompanying protocol shows that the repeated intravenous injection of as little as 100 mg. per kilogram of histidin caused serious renal injury.

PROTOCOL

EXPERIMENT 1—Rabbits 4 and 5, partly grown rabbits, were placed in adjoining metabolism cages in the laboratory, April 25, 1921. Rabbit 4, which weighed 1,400 gm. received repeated injections of histidin dichlorid. Rabbit 5, which weighed 1,700 gm., served as a control. Except for the injections, both rabbits lived under the same conditions. The urine of both rabbits was regularly examined on the same days. After three normal specimens had been obtained from each, Rabbit 4 began receiving the histidin, April 27. From that date until June 6, a total of forty days, he was given ten injections. The histidin dichlorid was dissolved in 5 c.c. of physiologic sodium chlorid solution and neutralized with sodium hydroxid against bromcresol purple as an indicator. The single dose calculated as histidin was 98 mg. per kilogram. The day after the first injection, the urine of Rabbit 4 was still normal. A few casts were seen after the second injection. Albuminuria did not become constant until after the fourth injection, from which time it slowly increased until the tests gave a heavy flocculent precipitate. Casts were always present after their first appearance and gradually increased in numbers until by May 20 they were recorded as very numerous. From June 6, when the last injection was given, until June 15 four specimens from Rabbit 4 continued to show much albumin and many casts. Throughout this whole period, thirty-one examinations of the urine of Rabbit 5 had invariably been negative. June 15, both rabbits were killed by

a blow on the head Rabbit 4 weighed 1,490 gm and his kidneys weighed 118 gm, or 79 gm per kilogram Rabbit 5 weighed 1,685 gm and his kidneys weighed 10 gm, or 59 gm per kilogram The kidneys of the treated animal, therefore, weighed 2 gm per kilogram more than those of the control

The histologic examination of a section of the kidney from Rabbit 4 showed multiple, delicate adhesions between the tufts and Bowman's capsule in the majority of the glomeruli, but there was no exudate in Bowman's space nor was the capsule thickened In the medulla casts were seen in many places The microscopic examination of the kidney of Rabbit 5 revealed no abnormalities

On the other hand, repeated injections of 60 mg per kilogram (Rabbits 6 and 7) were not productive of abnormal urine

Examination of the data obtained from the dogs shows that the kidneys of this carnivorous animal were slightly injured by repeated injections of 330 mg per kilogram (Dog 55 H), that two injections of 500 mg per kilogram caused greater injury (Dog 4 A), and that single injections of less than 15 gm per kilogram (Dogs 49 I and 61 K) caused abundant evidence of renal injury

Tyrosin—In order to get tyrosin into solution in the desired volume we used 10 c c of standard sodium hydroxid for each gram of the amino-acid Such solutions were alkaline to phenol red The question is therefore pertinent whether the alkalinity is of such a degree that it would in itself be capable of injuring the kidney, regardless of any effect of the tyrosin The alkalinity of a sample of the solution was measured with the potentiometer by L A Philipp of the department of physical chemistry, who found the p_H to be 10.61 Expressed in terms of normal alkali, the tyrosin solution has an alkalinity equal to two thousand five hundredth normal sodium hydroxid This very slight alkalinity would, on *a priori* grounds, not be expected to harm the kidney However, we have obtained experimental evidence that large amounts of sodium hydroxid of which the alkalinity has not been reduced in any way, such as is the case in the tyrosin solutions, gives no demonstrable evidence of renal injury

In a later section dealing with cystin, it will be noted that Dog 28 E received 336 c c of normal sodium hydroxid in the form of the free alkali This 84 c c per kilogram of normal alkali produced no evidence of injury This injection caused the dog to receive 134 gm of uncombined sodium hydroxid A like amount of tyrosin solution contains only 0.00054 gm of uncombined sodium hydroxid

The following experiment also demonstrates that large amounts of alkali may be injected into the vein of a dog without injuring the kidneys

EXPERIMENT 2—Dog 3 A, weighing 7,200 gm, Feb 20, 1923, at 1.30 p m was given morphin sulphate, one-quarter grain (0.01 gm), and at 2 p m, 34 c c of standard sodium hydroxid plus 0.8 gm of sodium chlorid made up to 100 c c with distilled water was slowly injected into a vein

February 21, the afternoon urine was slightly acid, there was no albumin, and casts were absent. February 23, 34 cc of standard sodium hydroxide prepared as on the previous occasion was injected into a vein. February 25, the afternoon urine showed no albumin and no casts. The first injection site presented a large area of necrosis. February 26, the urine showed no albumin and no casts.

Examination of Table 10 shows that definite evidence of renal injury is obtained following several injections of about 0.2 gm per kilogram of tyrosin into rabbits.

Larger single injections were followed by the prolonged presence of albumin and casts in the urine. The high values for blood nonprotein nitrogen obtained from Rabbits 20, 17 and 27 afford important evidence of renal injury. The reading of 66 mg obtained from Rabbit 27 nine days after the injection of 1.8 gm of tyrosin per kilogram is especially significant.

The dog experiments do not permit any conclusions regarding the smallest dose of tyrosin that will cause renal injury in them. Four of the seven dogs received 2 gm per kilogram of tyrosin, and in every instance that dose was productive of bloody urine. This fact is of special interest since tyrosin is the only one of the twelve amino-acids thus far studied which has caused red blood cells to appear in the urine.

The microscopic examination of the kidneys from the five dogs that were examined at necropsy, disclosed another feature of great interest. In three (Dogs 15, 23 C and 5 B) of these five dogs, the glomeruli contained important evidence of injury in the form of proliferation of the epithelial cells of Bowman's capsule. No other amino-acid gave us unequivocal evidence of glomerular damage. In the case of Dog 13, it was impossible to decide whether capsular proliferation due to tyrosin had occurred because so many glomeruli had been scarred by chronic kidney disease. The absence of signs of glomerular injury in Dog 22 C may be due to the short interval (twenty hours) between the injection and necropsy, or because this dog received only one injection, whereas the three dogs that showed the glomerular lesions had each received repeated injections over a number of days. Dog 15, whose protocol appears below, is instructive in this respect, since he was dead within twenty hours after the dose of 2 gm per kilogram, as was Dog 22 C twenty hours after its injection of 2 gm per kilogram. But Dog 15 had received four small doses of tyrosin preceding the 2 gm dose and showed the glomerular lesions, whereas Dog 22 C received only the one large injection and lacked the capsular proliferation.

EXPERIMENT 3—Nov. 8, 9 and 12, 1923, the urine of Dog 15, a puppy that weighed 5,600 gm, contained no casts or albumin. November 12, morphine sulphate, three-quarters grain (0.04 gm) was injected subcutaneously. One hour later, the animal was tied to a board and a vein in the hind leg was

TABLE 10—Typhoid

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 14	2,600	0.08 (0.2) daily, six in jections, after inter- val of eight days, 0.19 (0.5) daily, two injections	No casts were seen at any time	No necropsy	Killed by second injection of second series
Rabbit 16	?	(0.5) daily, two injections	Two specimens before injection, negative, urine, 18 hours after injection, negative	No necropsy	Killed by second injection
Rabbit 21	2,200	0.23 (0.5) every third day, five injections	Two specimens before injection, negative, con- stant albuminuria after first injection, casts first seen after fourth injection	No necropsy	Blood nonprotein nitrogen was 120 mg 24 hours after last injection
Rabbit 20	?	(0.5) every other day, four injections	Urine remained normal until after third injec- tion, when albuminuria became constant, casts were seen after fourth injection	No necropsy	
Rabbit 22	2,700	0.18 (0.5) every other day, three injections, after interval of ten days 0.36 (1) once	Two specimens before injection, negative, con- stant albuminuria after first injection, a few casts were seen after first injection, but not thereafter	No necropsy	Blood nonprotein nitrogen was 100 mg 48 hours after last injection, lethargic at this time, died in coma one hour later
Rabbit 17	1,500	0.66 (1) every other day, three injections	Urine normal before injection, constant albu- minuria after first injection, casts seen at every examination after third injection	No necropsy	
Rabbit 18	1,800	1.1 (2) once	Marked albuminuria 18 hours after injection, casts first seen three days after injection, they were present in every specimen for next eight days, thereafter, urine was normal	No necropsy	
Rabbit 27	2,300	1.8 (4) once	Four specimens before injection, negative, six hours after injection, albumin and a few casts were present, for the next nine days there was a heavy albuminuria, and many casts were seen in every specimen	Kidneys not preserved	Blood nonprotein nitrogen 9 days after injection was 68 mg per 100 cc of plasma

Dog 2 A	6,900	1 4 (10) once	Four specimens before injection, negative, two days after injection, urine contained much albumin and many casts, these abnormalities continued to be present for another 18 hours, thereafter, urine was normal	No necropsy	
Dog 22 C	2,150	2 (19) once	Two specimens before injection, negative, urine voided during 20 hours following injection was smoky owing to the presence of red blood cells, no casts seen	The lining cells of the convoluted tubules showed granular degeneration and swelling of nuclei, no coagulation in Bowman's space	Killed 20 hours after injection, blood nonprotein nitrogen 33 mg per 100 cc of plasma
Dog 1 A	3,300	0 9 (3) once, three days later, 15 (5) once	Urine showed albumin but no casts after first injection, 18 hours after second injection, much albumin and many casts were present, both were still found for another 18 hours, thereafter, urine was again normal	No necropsy	
Dog 15	5,600	0 5 (28) every other day, four injections, after interval of three days, 2 (37) once, after interval of three days, two injections 18 hours apart	Urine remained normal throughout the series of injections, urine voided during the 20 hours following injection of 2 gm per kilo, gram was pink, contained many red blood cells but no casts	Protocol of Dog 15 and Figure 1	Found dead 20 hours after last injection
Dog 23 C	1,850	0 5 (0 92) every other day, four injections, after interval of three days, 2 (37) once, after interval of three days, two injections 18 hours apart	Urine remained normal during first series of injections of 0 5 gm per kilogram, six hours after the injection of 2 gm per kilogram, pink urine, containing many red blood cells, was voided, during next 18 hours, many casts and red blood cells were present, urine obtained from bladder at necropsy contained many casts	Same changes as those described in detail in protocol of Dog 15 and illustrated in Figure 1	Died about 15 hours after last injection
Dog 5 B	2,150	1 (2 5) every third day, three injections after interval of 18 hours, one injection of 0 7 (1 7), 24 hours later, 2 (5) once	Casts first appeared after second injection of 1 gm per kilogram and continued to death	Same changes as those described in detail in protocol of Dog 15 and illustrated in Figure 1	Animal died at end of last injection
Dog 13	5,200	2 (10 1) once, after interval of three days, two injections 18 hours apart of 1 (5 2)	Two specimens before injection, negative, day after first injection, smoky urine containing many casts and red blood cells was obtained, urine continued to show many casts to death, 18 hours after last injection	Characteristic microscopic appearance of contracted kidney, this was the only adult dog used	Refused food for 18 hours after first injection, killed 48 hours after last injection, blood nonprotein nitrogen was 52 mg per 100 cc of plasma

* Figure in parenthesis is total single dose

exposed with the usual surgical precautions Tyrosin, 28 gm, was dissolved in 31 c c of standard sodium hydroxid on the water bath The solution was then brought up to 80 c c by addition of 0.8 per cent salt solution This solution was then allowed to flow slowly into the vein from a container enclosed in a jacket through which water at about 38 degrees C circulated The injection ended at 11 15 a m At 4 00 p m the dog voided urine containing neither albumin nor casts

November 13, the urine contained no albumin or casts

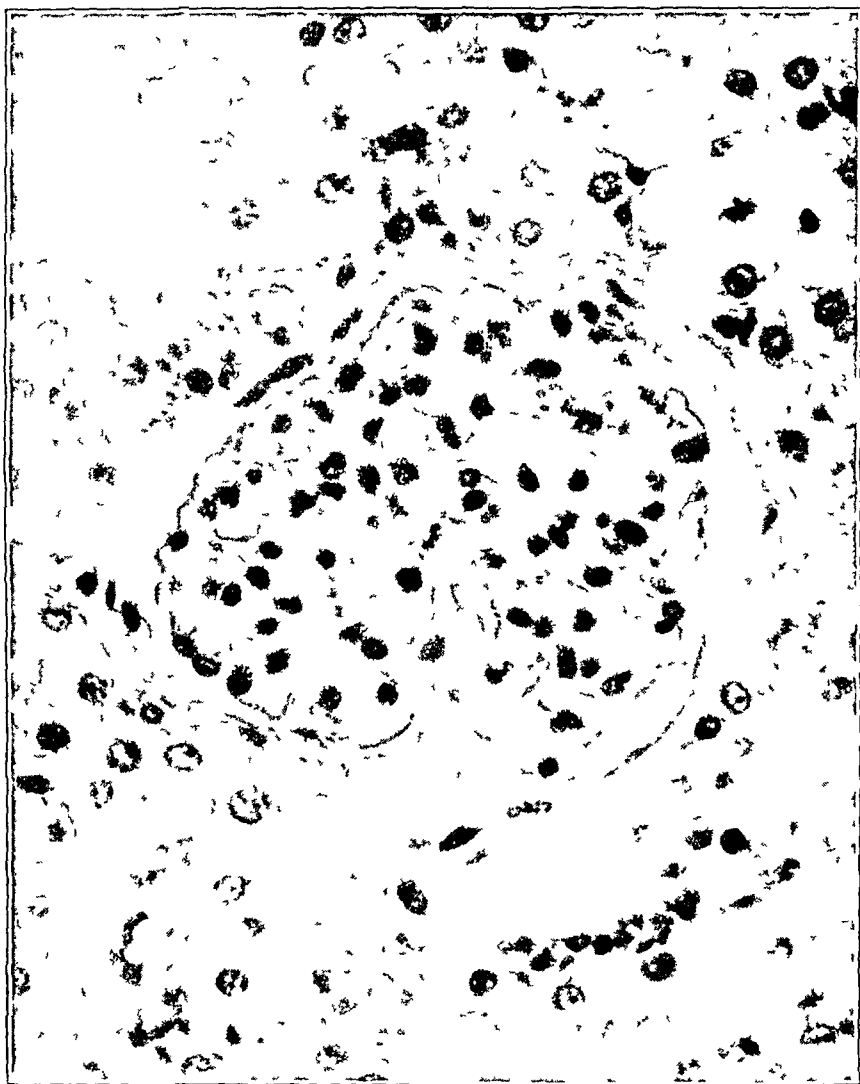


Fig 1—Cellular exudate in Bowman's space produced by intravenous injection of tyrosin into a dog

November 14, at 10 a m, 28 gm of tyrosin prepared as before was slowly injected into a vein one-half hour after the subcutaneous injection of morphin sulphate, three-fourths grain (0.04 gm) At 2 30 p m the urine contained no albumin but a very few casts

November 15 and 16, the urine was negative

November 17, at 10 a m, 28 gm of tyrosin was injected as before

November 18 and 19, the urine was negative

November 19, at 10 a m, 28 gm of tyrosin was injected as on previous occasions

November 20, the urine was normal

November 22, at 11 05 a m, 112 gm of tyrosin was dissolved in 120 c c of standard sodium hydroxid on a water bath and brought up to a volume of 225 c c with physiologic sodium chlorid solution This solution was allowed to flow into a vein in the course of seventy minutes The injection ended at 11 15 a m Toward the end of the injection, irregular twitching of the muscles of the face occurred At 9 p m the dog was quiet, and there was no twitching

November 23, at 8 30 a m, the dog was found dead in rigor mortis The urine collected during the night was pink (laked hemoglobin) There was a heavy albuminuria and the sediment contained many red blood cells but no casts

Necropsy—The lungs showed hypostatic congestion The organs other than the kidneys presented no gross abnormalities The kidney weighed 435 gm They were purple On section the surface was bathed in a serosanguinous fluid The urine obtained from the bladder was pink and contained much albumin, many red blood cells, some crystals of tyrosin, but no casts

Microscopic examination of a section of the kidney fixed in formaldehyd and stained with hematoxylin and eosin showed that the epithelium of the tubules in the cortex was stained irregularly The epithelial lining of the convoluted tubules, in particular, was swollen and granular, and the cell margins were no longer distinguishable Occasional short sections of tubules were seen in which the nuclei had disappeared and the cytoplasm had been changed into a mass of pink staining droplets occupying the lumen Many of the nuclei of the tubular epithelium were very small, structureless and intensely stained Nuclear fragmentations were common A few nuclei were swollen and pale Casts and desquamated epitheliums were plentiful Bowman's space in about half the glomeruli contained larger and smaller crescentic shaped and irregular collections of large plump epithelial cells whose nuclei were in varying states of preservation Some were quite pycnotic, others large and vascular The cytoplasm was granular and deeply stained It closely resembled in appearance the cytoplasm of the cloudy, swollen epithelial cells lining the convoluted tubules Lying around and between these cells was a homogeneous coagulum

Tryptophan—One gram of tryptophan will dissolve in 20 c c of physiologic sodium chlorid solution as the result of prolonged heating, but subsequent cooling to body temperature is quickly followed by precipitation Attempts to inject solutions of the foregoing concentration fail because the needle soon becomes plugged with crystals When 40 c c of salt solution is allowed for each gram of the amino-acid, little or no trouble from precipitation is encountered A dilution varying from 30 to 45 c c of salt solution for each gram of the amino-acid was used in the experiments summarized in Table 11 The resulting solution was neutral to phenol red

Examination of Table 11 leads to the impression that the kidneys of dogs are more easily damaged by tryptophan than those of the rabbit, but more experiments are needed definitely to answer this suggestion

The five dog experiments were very satisfactory since they displayed a steady increase in the extent of the injury proportional to the increasing size of the dose The injection of 0.5 gm per kilogram caused no general or local disturbance, but the animals that received 2.0 gm per

TABLE 11 — *Tryptophan*

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Rabbit 11	1,450	0.6 (1) once	Urine remained normal until 18 hours after injection, when a little albumin and a few casts were found, the next specimen was again normal	No necropsy	
Rabbit 13	1,750	1.1 (2) once	Negative before injection, urine collected during 20 hours following injection nearly black (melanin?), and showed heavy albuminuria and many granular casts	No histologic examination of kidney	Found dead 30 hours after injection, left lung congested but air containing
Rabbit 16	1,550	1.2 (1.8) once	Urine remained normal during the 48 hours following injection	No necropsy	
Dog 47 II	4,300	0.5 (2.15) once	Four specimens before and three after injection were all normal	No necropsy	
Dog 54 K	2,600	0.85 (2.2) once	Two specimens before injection, negative, 24 hours after injection, urine contained a little albumin and a few casts, thereafter, it was normal	No histologic abnormalities seen	Killed third day after injection
Dog 44 G	3,350	1 (3.5) once	Two specimens before injection, negative, specimens 24 and 48 hours after injection showed many granular casts and renal tubule cells	The lining cells of the convoluted tubules took stain irregularly and many nuclei were pyknotic, many casts in tubules	Killed 48 hours after injection
Dog 32	1,950	2 (4) once	No urine voided after injection, bladder empty at necropsy	Widespread simple necrosis of convoluted tubular epithelium, many casts (Fig. 2)	Animal in coma the morning after injection, killed
Dog 43 G	2,850	2 (5.7) once	Two specimens before injection, normal urine for 36 hours following injection, urine found in bladder at necropsy contained enormous numbers of granular casts	Figure 2 Experiment 4	Refused food for 36 hours after injection, killed 45 hours after injection
Rabbit 45	1,900	1 (2) once	Experiment Not Acceptable Hematuria following injection		Killed 24 hours after injection, ecchymoses of bladder mucosa, capillary bleeding

* Figure in parenthesis is total single dose

kilogram suffered from a serious general intoxication, and a massive destruction of the renal tubules occurred in as short a time as thirty hours (Dog 32)

The protocol of Dog 43 G and Figure 2 give the details of an experiment in which a dog received a single injection of 2 gm per kilogram

EXPERIMENT 4—The urine of Dog 43 G, a puppy, contained no albumin and no casts, April 27 and 28, 1924 The dog weighed 5,600 gm

April 28, at 2 p m, morphin sulphate, three-fourths grain (0.04 gm), was injected subcutaneously About one hour later, we began the injection of a solution of tryptophan made by dissolving 57 gm of tryptophan in 230 c c of physiologic sodium chlorid solution on a water bath at a temperature of from 38 to 40 degrees C The injection took forty-five minutes and ended at 3 45 p m

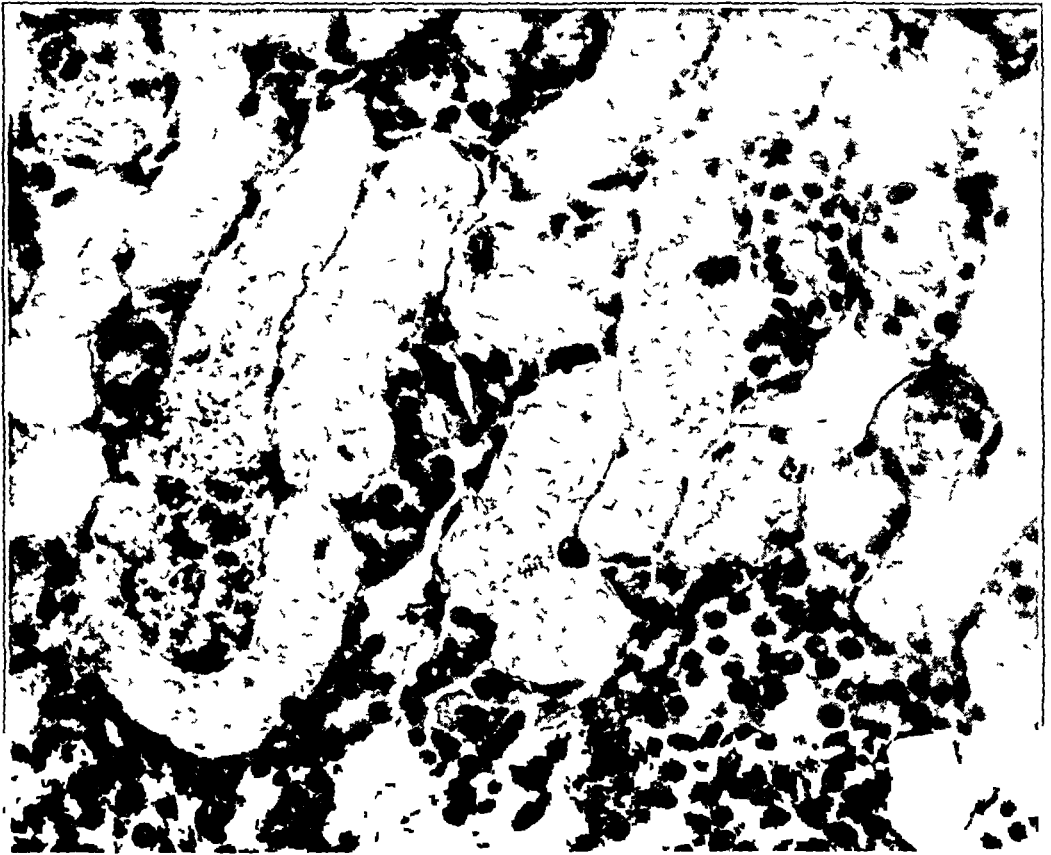


Fig 2—Widespread necrosis of the renal tubules produced by intravenous injection of tryptophan into a dog

The morning of April 29 the dog looked sick, he lay on the bottom of the cage and refused food No urine had been voided during the night

April 30, the dog was still sick, and had not moved about The morning urine was contaminated At noon he was killed by a blow on the head

Necropsy—The kidneys weighed 30 gm The capsule stripped easily leaving a smooth surface, gray in color, except for the presence of several ecchymotic patches The section surface was gray, moist and lacking in markings, except those made by the arteries of the cortex Several of the ecchymotic patches extended deep into the cortex The latter appeared to be wider than normal

The other organs presented no gross abnormalities. The urine contained in the bladder was free of albumin, but the sediment contained an enormous number of granular and cellular casts, and desquamated tubular epithelium.

Microscopic examination of the kidney showed that the majority of the cortical tubules had been severely injured. The nuclei of the lining cells of such tubules had either entirely disappeared, or had, in a few cells, been changed into smooth, structureless, deeply stained points. The cells in most of the cortical tubules had lost their margins, had become detached from the basement membrane, and lay free in the lumina as homogeneous, compact, smooth, deeply stained bodies (casts), or as paler, coalescing, granular droplets crowded together in the lumen. Some of the tubules, entirely denuded of epithelial lining, were empty. The glomeruli showed no injury. As one passed into the medulla, the cellular injury became less marked. The tubules of the medulla appeared to be normal, but contained many casts and desquamated epitheliums. The picture was that of widespread necrosis of the convoluted tubules.

Cystin—This went into solution when 8.2 cc of normal sodium hydroxid was used for each gram. The resulting solution was slightly alkaline to phenol red. Evidence has already been presented in the section devoted to tyrosin which shows that the alkalinity of the solution could not be held responsible for the interesting renal injury produced by cystin. Further evidence of the truth of this statement is obtained by an examination of the data from Dog 28 E.

EXPERIMENT 5—Dog 28 E, weighing 4,000 gm, received an intravenous injection of 33.6 cc of normal sodium hydroxid diluted to a volume of 100 cc with salt solution, February 14. The urine collected from the time of the injection to 9 a. m., February 15, contained no albumin and no casts. A second specimen of urine obtained the afternoon of February 15 and the urine voided the morning of February 16 failed to show either albumin or casts.

February 18, at 3.30 p. m., the dog received an intravenous injection of 4 gm of cystin dissolved with 33 cc of normal sodium hydroxid and diluted to a volume of 100 cc with physiologic sodium chlorid solution.

February 19, at 5 p. m., the dog passed urine that contained an abundance of cellular casts and desquamated renal tubule cells. Albumin tests were negative. Urine collected during the morning of February 20 contained many cellular and granular casts and large granular cells but no albumin.

February 22, similar urine was obtained. February 25, the urine was again normal.

Examination of Table 12 shows that a single injection of 0.5 gm per kilogram of cystin will produce evidence of renal injury.

The effect of somewhat larger doses is described in detail in the protocol of Dog 31 E. Figure 3 shows the character of the lesion in the renal tubules.

EXPERIMENT 6—Dog 31 E, a puppy weighing 4,400 gm, was used. The urines of March 1 and 3, 1924, contained neither albumin nor casts.

March 3, at 3.30 p. m., the dog was given a subcutaneous injection of morphin sulphate, one-half grain (0.03 gm). At 4.30 p. m. 3.5 gm of cystin dissolved in 29 cc of normal sodium hydroxid and brought up to a volume of 75 cc with physiologic sodium chlorid solution was allowed to flow slowly into a vein previously exposed with aseptic precautions.

March 4, a flocculent precipitate formed in the boiled urine. It did not disappear on the addition of dilute acid. The cold urine to which dilute acetic acid was added also yielded a heavy precipitate. It was thought that this latter precipitate was cystin. Acetic acid was accordingly added to a portion of the urine until the filtrate remained clear on the addition of acid. This filtrate gave strongly positive tests for albumin. The sediment contained many desquamated epithelial cells, cellular and granular casts.

March 5, the urine gave less marked tests for albumin. The sediment was similar to that of the previous day.

March 7, 8 and 10, numerous cellular, granular and hyaline casts were seen in each specimen of urine.

March 12 and 13, the urine was albumin free, and no casts were seen.

March 14, the dog weighed 6,500 gm. Cystin, 6.5 gm, prepared as on March 3, was allowed to flow slowly into a vein about one hour after the dog had received morphin sulphate, three-fourths gram, subcutaneously.

March 15 and 16, in addition to albuminuria many epithelial cells, large cellular, granular and hyaline casts were present in both specimens.

March 16, at 3.30 p. m., the dog was killed by a blow on the head. At necropsy the kidneys weighed 78 gm. When the capsule was stripped off a pale yellow ground over which were strewn red points singly and in groups and irregular red patches was disclosed. Toward the hilum of one kidney a broad patch of cyanotic discoloration was seen. The section surface presented the same variegated appearance. No markings but the straight arteries were seen in the cortex. The other organs appeared normal.

Microscopic examination of the cortex of the kidney revealed many small areas that stood out sharply because of the lavender-blue presented by the irregular groups of structureless, smooth droplets occupying the space of a tubule. Higher magnification showed that such areas consisted of a section of tubule in which the epithelial cells had disappeared as such and had been changed into dead protoplasm impregnated with calcium salts. In some of these tubules a transition could be seen from fairly normal cells at one end through cells whose nuclei were greatly swollen, lacking in chromatin, and sometimes were represented by only a blue ring and whose cytoplasm was stained pale blue to masses that had lost all resemblance to cells and had entirely replaced the tubular structure in the places where they occurred. Such areas of calcification were being surrounded by fibroblastic cells often several layers deep. Many tubules showed the earlier stages of this process but their epithelial cells had not yet lost their nuclei. Other tubules were stained a deep pink and consisted of cells with pale nuclei and granular, swollen cytoplasm. Desquamated cells and casts were numerous. In a few lumina small clumps of red blood cells were seen. The glomeruli were engorged and appeared to contain more nuclei than normal. The medulla presented no definite abnormalities.

It is an interesting question whether the renal injury caused by the intravenous injection of cystin and some of the other amino-acids would also occur if they entered the body through the liver, where they might be changed into nontoxic substances, or whence they might be slowly distributed in dilutions so great as to be innocuous. A single experiment gave us a clear answer to this question regarding cystin. The data obtained from Dog 39 F, Table 13, showed that the injection of 1 gm of cystin per kilogram into a tributary of the portal vein was followed by the urinary signs of renal injury.

TABLE 12 — Cystin

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine	Histology	Remarks
Dog 60 K	5,200	0.5 (2.6) every other day, three injections	Two specimens before injection, negative, a few casts seen daily after first injection, they gradually increased until they were "frequent" after third injection	No histologic abnormalities of kidney seen	Killed 45 hours after first injection
Dog 29 E	3,270	0.5 (1.65) every third day, five injections	Two specimens before injection, negative, after second injection, a few granular and hyaline casts found at every examination	Right kidney epithelium of convoluted tubules overstained, swollen and hazy, in a few tubules the cells had lost their nuclei and the cytoplasm was represented by pale droplets filling the lumen Proximal convoluted tubules show moderate cloudy swelling, no casts	Killed 48 hours after last injection, left kidney presented the picture of congenital hydronephrosis
Dog 38	1,750	1 (1.75) once	Two specimens before injection, negative, specimen day after injection, negative		Killed 30 hours after injection
Dog 28 E	4,000	Note 33.6 cc of normal sodium hydroxid brought up to 100 cc volume with salt solution, four days later, 1 (1) cystin plus 33.6 cc of normal sodium hydroxid brought up to 100 cc volume with salt solution, eight days later, cystin injection repeated	Three specimens before injection, negative, three specimens following injection of alkali, negative, 48 hours after first injection of cystin many granular and cellular casts seen, seven days after this injection, urine again normal, findings after second injection of cystin not significant because of the pneumonia	Histologic abnormalities not significant because of pneumonia	Killed 48 hours after second injection of cystin, killed necropsy diagnosis, pneumonia
Dog 31 E	4,400 at time of first injection, 6,500 at time of second injection	0.8 (3.3) once, eleven days later, 1 (6.5) once	Two specimens before injection, negative, many casts and tubule cells found day after first injection, present daily for ten days, absent day after second injection, thereafter, present to death	Experiment 6, Figure 3	Killed 48 hours after second injection

Dog 37	1,150	1 (1.15), two injections with interval of six days	Two specimens before injection, negative, 48 hours after first injection, the urine contained much albumin, many granular and some hyaline casts, 48 hours after second injection, the urine showed similar abnormalities	Convoluted tubules stood out distinctly because they were stained a much deeper pink than rest of tissue, epithelial cells of these tubules showed signs of degeneration swelling, loss of outline, vacuolation, pyknosis and disappearance of nuclei, but no calcification seen glomeruli apparently normal, casts very numerous	Killed 48 hours after second injection
Dog 24 C	2,650	1 (2.65), two injections with interval of six days	Three specimens before injection negative, five hours after first injection, albumin and many granular and hyaline casts found, 24 hours after first injection, sediment contained enormous numbers of large granular mononuclear cells and many casts showing all transitions from a series of several epithelial cells, whose borders were still plainly seen, to typical granular casts, similar urinary abnormalities were noted thereafter until death	Microscopic picture same as that described in protocol of Dog 31 and illustrated by Figure 3	Killed 24 hours after second injection
Dog 27 D	2,750	2 (5.5), two injections with interval of nine days	Two specimens before injection, negative, 24 hours after injection, no casts 72 hours after injection, in my granular and some cellular casts, similar sediment found regularly there after	Histologic changes of same type as those found in the kidneys of Dogs 24 C and 31 E but more extensive, many more tubules showed advanced degenerative changes, while calcification was marked, it had taken place in only a minority of the injured tubules broad patches of hemorrhage seen in upper medulla, extreme congestion seen everywhere	Died 6 hours after second injection, necropsy all organs engorged, scattered irregularly over peritoneal surfaces were white flecks easily picked up in form of dry curdlike material, pleural surfaces presented same features, when capsule of kidney was stripped off, dull red background was seen over which minute white points were dusted, on section, curdlike material was found in renal pelvis veins stood out as broad black bands, collecting tubules in medulla were visible as intense white streaks, cortical tissue was streaked by fine white lines
Dog 35	1,750	0.25 (0.5) once	Urine remained normal during 48 hours following injection	Five days after injection, diarrhea	Continued because of diarrhea
Dog 34	2,800	1 (2.8) once	Two specimens before injection, negative	Died 48 hours after injection on necropsy revealed extensive pneumonia	

* Figure in parenthesis is total single dose

Dog 40 F received the same treatment as its litter mate, Dog 39 F, except that the solution injected into the former contained no cystin. The continued absence of casts and other abnormalities from the urine of Dog 40 F after the injection proves that neither the anesthetic nor the technic of the operation can be held responsible for the signs of kidney injury which followed the injection of cystin into a portal tributary of Dog 39 F.

CONTROLS

The experimental procedure used in these studies was so simple that the chance of error inherent in the employment of elaborate appa-

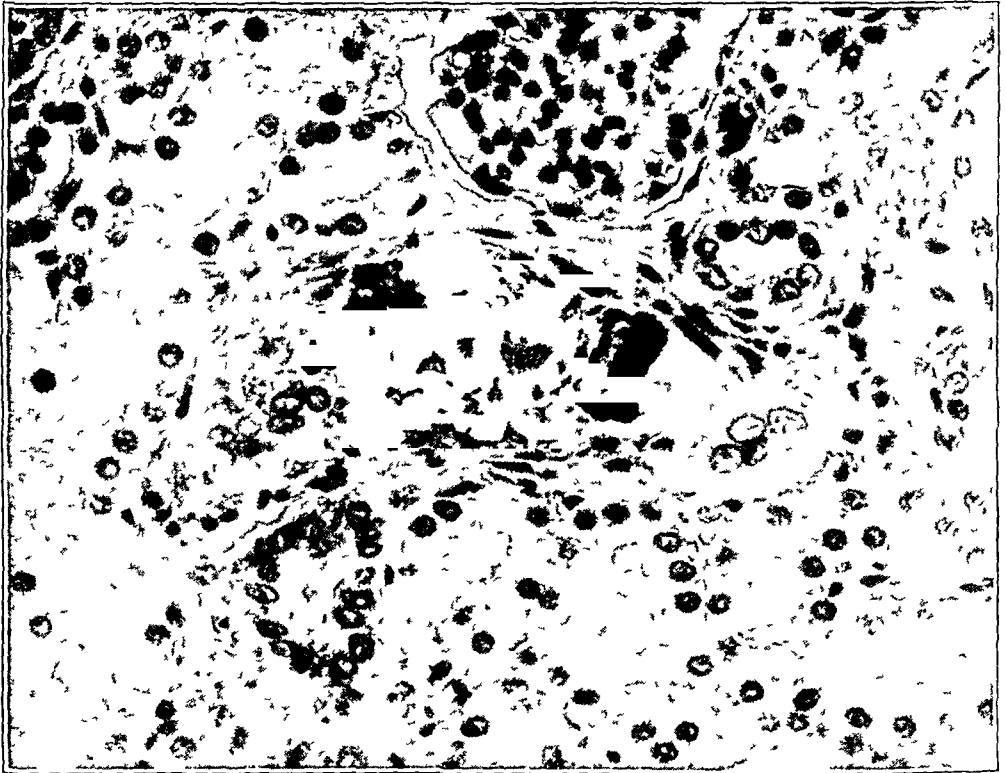


Fig 3—Necrosis and deposition of calcium salts in some of the dead cells following the intravenous injection of cystin into a dog

ratus and complicated technic was almost negligible. The safety of the intravenous route has been so thoroughly established by its very extensive use both clinically and experimentally that disturbances following this method of administration are confidently attributed to the injected substance and not to the technic. Nevertheless, it is desirable to control this procedure as used by us. The danger of error, if any, lies in the bulk of the solution and its reaction, in struggling on the part of the animal, in the anesthetic used to avoid it, and finally in infection of the injection site.

That none of these things was the cause of the renal damage in the case of the amino-acids that we have concluded are nephrotoxic is proved by the uniformly negative results obtained with alanin, leucin, glycin, phenylalanin and glutamic acid. For example, the dog that was given leucin so diluted that he received 82 c c of fluid per kilogram manifested no subsequent renal disturbance. Likewise, large amounts of alkali were required for the neutralization of glutamic acid and the latter was given to Dog 57 K with 48 c c of combined standard sodium hydroxid, or more than 14 c c per kilogram, without renal damage (glutamic acid section). Even more impressive is the experience with Dog 25 D (Table 7), who received a total of 193.6 c c of standard sodium hydroxid in six days, used to neutralize aspartic acid, and who continued to void normal urine. In the case of these amino-acids, the alkali was used to neutralize the acidity of the dissolved amino-acid, but tyrosin and cystin require the solution to be slightly alkaline to maintain solubility, hence the negative results obtained with the large amount of alkali injected as a part of a neutral solution do not completely control possible renal injury from the alkaline quality of solutions of tyrosin and cystin. It has been pointed out that in the case of tyrosin the alkalinity amounted to only two thousand five hundredth normal (Table 9). The cystin solution was of the same order of alkalinity. Furthermore, the administration of 34 c c of free standard sodium hydroxid twice to one dog (tyrosin section) and 33.6 c c of free sodium hydroxid to another dog (cystin section) was not followed by any evidence of renal damage. Since these large amounts of free alkali had no harmful effect on the kidneys, it is evident that the nephrotoxicity of the solutions of tyrosin and cystin is solely attributable to the dissolved amino-acid.

Violent struggling is known to produce albumin and casts in the urine. The rabbits offered no resistance to the injections. Struggling was satisfactorily prevented in the dogs by the use of morphin. It is clear that this drug does not damage the kidneys because of the negative results following its use in the case of the five amino-acids already mentioned, and in the control experiments with alkali.

Infection at the site of administration never occurred in rabbits because there was incision. In the dogs the wounds nearly always healed normally. In a few instances the wounds did not heal by first intention, but the largest sloughs occurred in the animals that received the free alkali.

It might be thought that the excretion of the large amount of urea formed from the injected amino-acids caused the signs of renal injury noted by us. That this is not an adequate explanation is shown by the fact that some amino-acids did not injure the kidneys. It will be recalled that the innocuous amino-acids are, in general, those of smaller

TABLE 13.—*Injection of Cystin into a Mesenteric Ven*

Animal	Weight, Gm	Dose per kilogram,* Gm	Urine	Histology	Remarks
Dog 39 F	3,900	1 (3.9) once neutral ized with 32 c c of nor mal sodium hydroxid and made up to vol ume of 100 c c with salt solution	Two specimens before injection, negative, 20 hours after injection, no cists or red blood cells, 24 hours after injection, several large granular cists and some red blood cells found, 40 hours after injection, numerous granular cists and occasional red blood cells seen Two specimens before injection, negative, two specimens following injection, negative	Epithelium of convoluted tubules showed moderate cloudy swell ing, typical cists numerous	Ether used for anesthesia, good recovery, killed 48 hours after operation, oper ation site clean
Dog 40 F	3,700	100 c c of salt solution		No histologic abnormalities of kidney	Ether used for anesthesia good recovery, killed 48 hours after operation, no signs of infection

* Figure in parenthesis is total single dose

molecular weight, and that they therefore actually yielded more urea gram for gram than the nephrotoxic amino-acids. The injections of urea given in Table 14 afford clear evidence that it is not damaging to the kidneys.

COMMENT

The intravenous administration of alanin, leucin, glycine, phenylalanin and glutamic acid gave us no evidence of renal injury in doses as large as 2 gm per kilogram. Arginin and aspartic acid are mildly nephrotoxic, while serious kidney damage was produced by lysin, histidin, tyrosin, tryptophan and cystin. We have been unable to obtain serum or prolin.

The most important outcome of these studies is the demonstration that normal digestion products of protein are a source of renal injury under the conditions of these experiments.

TABLE 14—Urea

Animal	Weight, Gm	Dose per Kilogram,* Gm	Urine
Rabbit 25	2,500	2 (5) dissolved in 30 cc of salt solution	Urine normal before injection, 24 hours after injection catheter specimen con- tained no albumin, 60 hours after injection, catheter specimen contained no albumin
Rabbit 27	2,200	2.3 (5) dissolved in 20 cc of physiologic sodium chlorid solution	Two specimens before injection, negative, 4 hours after injection, urine nor- mal, 24 hours after injection, urine contained no albumin and no casts
Rabbit 28	1,850	2.8 (5) dissolved in 20 cc of physiologic sodium chlorid solution	Two specimens before injection, negative 24 hours after injection, urine con- tained neither albumin nor casts 48 hours after injection, urine con- tained neither albumin nor casts 60 hours after injection urine con- tained neither albumin nor casts

* Figure in parenthesis is total single dose

It will be recalled that this investigation was undertaken in an attempt to explain the renal damage from high protein diets, since such diets increase the absorption into the blood stream of amino-acids. The concentration of nephrotoxic amino-acids following a high protein meal probably does not reach that caused by our injections. The same thing is true of the amino-acids and their metabolites in the urine. But it is also in general true that repeated doses of a poison, each too small to cause demonstrable injury, will eventually result in serious damage. May there not be a similar relationship between the chronic nephropathy from high protein diets and the acute injury following the injection of amino-acids? We have noted in the case of some amino-acids that single doses that were too small to give evidence of injury would cause the appearance of abnormal urine, if repeated only a few times. Furthermore, we do not know how small a dose of injected amino-acid will

cause injury. The crudity of the methods for detecting such injury precludes the possibility of demonstrating it in lesser degrees. This difficulty became very evident to us when we found that it requires about twice as much amino-acid to give unequivocal histologic evidence of renal damage as is needed to produce cylindruria. The smallest doses with which we obtained abnormalities are given in Table 15.

The amino-acids normally enter the body from the digestive tract, hence it may properly be questioned whether information obtained by intravenous injection has any bearing on effects attributed to excesses arriving through normal channels. A certain amount of information tending to answer this question is available. Our injection of cystin into the portal vein of Dog 39 F shows that the injury is not prevented by passage of the amino-acid through the liver. In certain investigations of the metabolism of cystin, H. B. Lewis⁸ (1925) has noted that the ingestion of cystin by rabbits is followed by serious kidney damage.

TABLE 15—*Smallest Dose of Amino-Acid that Produced Renal Injury*

Amino Acid	Rabbits, Gm per Kg	Dogs, Gm per Kg
Arginin	1.0	
Aspartic acid	0.5	2.0 (?)
Lysin	0.15	0.6
Histidin	0.10	0.33
Tyrosin	0.23	1.0
Tryptophan	0.6	0.85
Cystin		0.5

Finally, experiments still incomplete being conducted by A. C. Curtis have shown that the addition of cystin to standard diets causes renal injury in rats.

It is also of interest to note the difference in the reactions of the kidneys of rabbits and of dogs to some of the amino-acids. These differences are to be seen in Table 15.

Sufficient information is not at hand to assign a place to these new data in the problem of the etiology of chronic nephritis in man. The difficulty is in part due to the difference in reactions of different species to the amino-acids, and in part to the difference in the histologic picture produced by the amino-acids and that found in the human cases. Nevertheless, the fact remains that certain amino-acids are capable of producing serious injury of the kidney. Unless future investigators show that this fact is irrelevant, it must be included in any attempt to formulate a comprehensive picture of the etiology of human nephritis.

⁸ Lewis, H. B. J. Biol. Chem. 63: 20, 1925.

In this connection it is of interest to mention that Folin and Berglund⁹ (1924) have found another normal product of protein metabolism that causes serious renal disease. In the course of their studies of uric acid metabolism they found that the intravenous injection of uric acid was attended by evidence of renal injury.

SUMMARY

It has been demonstrated that the amino-acids, arginin, aspartic acid, lysin, histidin, tyiosin, tryptophan and cystin are nephrotoxic

⁹ Folin, O , Berglund, H , and Derick, C J Biol Chem **60** 301 (June) 1924

THE AURICULAR WAVE (P) OF THE ELECTRO-CARDIOGRAM

CLINICAL OBSERVATIONS WITH ESPECIAL REFERENCE TO PULMONIC
AND MITRAL STENOSES

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LITERATURE

Studies concerning the diagnostic significance of changes in the auricular or P wave of the electrocardiogram have not been numerous. Increase in height and prolongation of the wave, occurring most often in mitral stenosis, have been noted by Einthoven,¹ Kraus and Nicolai² and Samojloff,³ Goddard⁴ assumed a more or less definite quantitative relationship to exist between such augmentation of the P wave and auricular hypertrophy. He considered as increased all P waves when they were greater than T in the same cuive, that is $\frac{P}{T} = 1 +$. Checking this relationship, he found that there was some relative increase in height (of the P wave) in 54 per cent of the cases of mitral disease studied. However, in five cases that came to necropsy, one with very slight hypertrophy of the auricle showed marked exaggeration of the P wave while another with decided hypertrophy showed only a slight relative alteration of the P. On the other hand a check of all the exaggerated P waves against the histories of the patients showed that 45 per cent had mitral disease and that a small percentage had clinically normal hearts. Goddard then concludes that the association of P wave exaggeration with auricular hypertrophy, though suggestive, is not a constant one.

Experiments seeking to determine the nature and source of the P wave by Samojloff,⁵ Kraus and Nicolai,⁶ Lewis, Meakins and White,⁷

1 Einthoven, W. Le Telecardiogramme, Arch internat de physiol 4 148, 1906-1907

2 Kraus, F, and Nicolai, G F. Berl klin Wchnschr 44 812, 1907

3 Samojloff, A. Munchen med Wchnschr 56 1943, 1909

4 Goddard, C H. Arch Int Med 16 633 (Oct) 1915

5 Samojloff, A. Festschrift Herman, 1908, p 171

6 Kraus, F, and Nicolai, G F. Das Elektrokardiogramm des Gesunden und Kranken Menschen, Leipzig, 1910

7 Lewis, T, Meakins, J, and White, P D. Phil Tr Roy Soc London, 205 375, 1914

and Eyster and Meek⁸ have shown that it is a composite representation of the excitation wave as it spreads from the region of the sinus, the sino-auricular node, over the auricle. Recently, the interesting and fundamentally important work of Einthoven⁹ has shown that the electrical and mechanical expressions of muscular activity are synchronous and parallel in degree. This applies to heart muscle as well as to skeletal muscle. The myocardiogram if accurately obtained corresponds exactly with the electrocardiogram in time of onset, duration and force. Thus the auricular or P wave of the electrocardiogram does not represent simply the brief early expression of the excitatory process in the auricle but in fact the entire auricular contraction. Instead of measuring in time only one third of the duration of auricular action it really coincides with it. The T wave of the auricle—the so-called Ta wave of the electrocardiogram—had been pointed out years ago by several observers but it has now been correlated to auricular systole by Einthoven⁹. Sprague and White¹⁰ have recently shown the frequent clinical occurrence of this Ta wave of the electrocardiogram. Thus, the larger and more active the auricles, the larger and longer should be the auricular deflections of the electrocardiogram.

Attention has also been called to the influence of the position of the heart in the body, as determined by respiration or other factors, and of the periodic shift of the pacemaker within the node, as shown by Lewis, Meakins and White⁸.

Finally, the influence of the extrinsic cardiac nerves on the P wave has been studied. Einthoven¹¹ demonstrated that simple section of the vagus in dogs produced a marked rise in the P wave. In such an experiment of course changes in the auricle could play no rôle. Rothberger and Winterberg¹² repeated and confirmed this, and further showed that section of the cardiac accelerators (increased vagus effect) was followed by a pronounced decrease in P. Selenin,¹³ however, saw no such effect on the dog's heart in situ, following full therapeutic dosage of digitalis.

PRESENT INVESTIGATION

The reason for the present study was the desire to determine the significance of high P waves with reference to the diagnosis of pulmonary stenosis and mitral stenosis.

8 Eyster, J. A. E., and Meek, W. J. *Arch. Int. Med.* **11**: 204 (Feb.) 1913.

9 Einthoven, W. *Goddard Lectures*, Harvard Medical School, 1924.

10 Sprague, H. B., and White, P. D. *J. Clin. Invest.* **1**: 389 (April) 1925.

11 Einthoven, W. *Arch. f. d. ges. Physiol.* **122**: 537, 1908.

12 Rothberger, J., and Winterberg, H. *Arch. f. d. ges. Physiol.* **135**: 506, 1910.

13 Selenin, W. P. *Arch. f. d. ges. Physiol.* **143**: 137, 1912.

The study has included three distinct parts as follows First, the analysis of (a) the amplitude of the P wave, (b) the values of angle and index of electrical axis deviation, and (c) the clinical diagnosis in 132 unselected cases seen at the Massachusetts General Hospital from 1914 to 1924 in whom electrocardiograms showed an amplitude of P in Lead I greater than 0.1 millivolt (1 mm), or of P in Lead II equal to or greater than 0.3 millivolt (3 mm) Second, a similar analysis of all cases definitely diagnosed pulmonic stenosis and coming to electro-

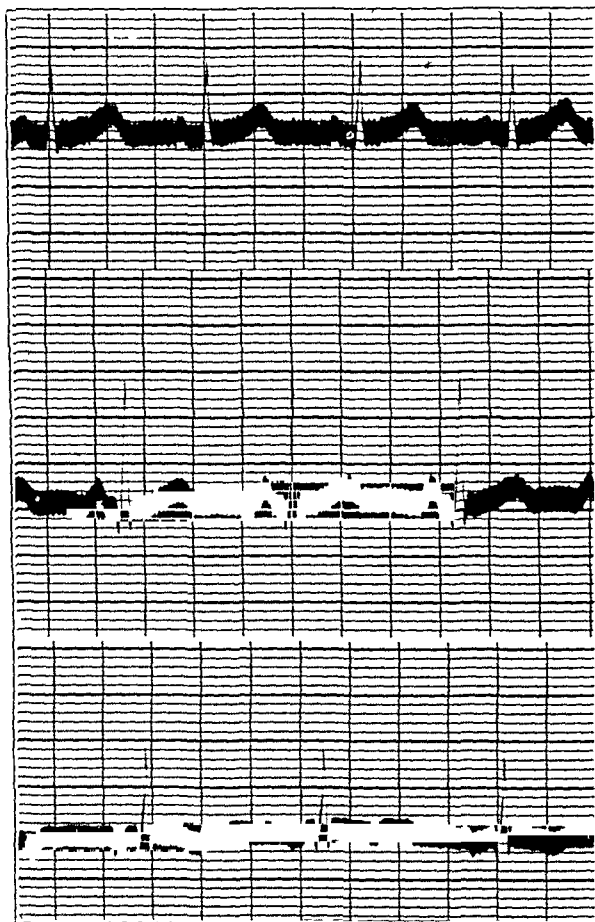


Fig 1—Leads I, II and III, normal, for comparison with Figures 2 and 3, time, 0.2 second, potential, 10^{-4} volts per millimeter

cardiographic study at the Massachusetts General Hospital from 1914 to 1924 Third, the same study of 100 unselected cases of "pure" mitral stenosis electrocardiographed at the Massachusetts General Hospital

The height of the P wave has been studied rather than its length or duration for several reasons First, it is more evident and more easily measured, second, the occurrence of the T wave of the auricle, the so-called Ta wave, sometimes appearing as a direct prolongation

of the P wave, makes it difficult to determine the limits of the P, and third, the height of the P wave may be abnormally increased with little if any increase in duration while the reverse is rarely true. Almost invariably if the P is longer than normal it is also higher than normal in either Lead I, Lead II, or both. For example, out of the fifty-eight cases of mitral stenosis with normal rhythm (listed in Table 7) only nine failed to show high P waves in either Lead I or Lead II and none of these nine showed any abnormal length of the P waves, incidentally, the P wave in Lead III was low in all of these nine cases.

Rarely if ever is the P wave in Lead III as large as in the other leads, usually it is of very low potential, either upright, inverted or isq-electric. Thus P_3 has not been included in the present analysis because of its unimportance.

With the abnormal increase in height of the P waves in Lead I and Lead II there is often abnormal notching in addition to increased length. A duration of the P wave (not including the Ta wave) beyond 0.1 second in any lead and a height above 1 mm (0.1 millivolt) in Lead I, or a height equal to or above 3 mm (0.3 millivolt) in Lead II, we have considered abnormal.

The formula used for determining the index was that outlined by White and Bock¹⁴ and the angles were arrived at by the use of the triangle of Einthoven, following the method described by Carter, Richter and Greene¹⁵. By the index method figures of -12 or more negative indicate abnormal right axis deviation, and of $+20$ or more positive abnormal left axis deviation, -12 to $+20$ are within normal limits. By the angle method figures beyond $+90$ degrees represent abnormal right axis deviation and on the negative (upper) side of 0 degrees abnormal left axis deviation, 0 to $+90$ degrees (the right lower quadrant) are within normal limits.

PART I

Tables 1, 2, 3, 4, 5 and 6 include the cases examined in the first part of the study. The clinical diagnosis has determined the inclusion of the various cases under one or another of these tables, wherein pulmonary stenosis, mitral stenosis pure, and mitral stenosis with other valve defects are each given separately, while other diagnoses encountered are embodied in Table 6.

Examination of Tables 2, 3, 4 and 5 shows that the amplitude of P_2 was equal to or greater than 3 mm in 68 per cent of all of the seventy-two cases of mitral stenosis encountered, and an interesting

¹⁴ White, P. D., and Bock, A. V. *Am J M Sc* **156** 17 (July) 1918.

¹⁵ Carter, E. P., Richter, C. P., and Greene, C. H. *Bull Johns Hopkins Hosp* **30** 162 (June) 1919.

TABLE 1—*Pulmonic Stenosis* *

Plate No	Index, Mm	Angle, Degrees	P ₁ , Mm	P ₂ , Mm
6413	-20	150	2.5	4
7465	-30	90	1	5
6838	-34	125	2	4
1994	-12	165	1.8	3.8
7919	-42	159	4	4
745	-43	143	1.5	1.2
3940	-18	112	2	2.5
6448	-36	134	2	3
5805	-28	150	2	4.5
974	-25	140	2	2.5
4057	-23	142	2.5	3
6573	-32	132	1	3
7465	-30	90	1	5
7438	-24	118	2	3.5
5879	-30	143	1.7	4
7050	-18	149	2	2.5
6590	-28	118	2	3
8870	-16	116	1.5	4
6448	-36	134	3	4
8098	-29	118	0.5	6

* Values throughout these tables are positive unless otherwise noted

Summary Total cases, nineteen (twenty plates) Sixteen cases had an amplitude of P₂ equal to or greater than 3 mm. The other three cases had an amplitude of P₁ greater than 1 mm. Eighteen had an index below -15. One had an index of -12. All had angles of 90 degrees or more.

TABLE 2—*Mitral Stenosis (Pure)* *

Plate No	Index, Mm	Angle, Degrees	P ₁ , Mm	P ₂ , Mm
905	-15	120	3	3
1917	-11	69	2	2
2209	-17	102	2	2
2275	-15	130	1.8	2
1605	-8.5	81	1.5	2.7
3095	-13	95	1	4
2352	-19.5	120	2.2	2.2
2362	2	56	1.5	3.5
6653	-1	59	2	3
6685	-20	90	1	3
6675	-1	62	2	3.5
6801	-7	75	1.5	3.5
6774	-4.1	164	1	3
6865	-6	90	2.2	1.8
6879	-23	102	1.2	2.5
6959	-10	72	1.5	3
6948	5.5	25	2	4
8518	-12	98	2	5
7151	-5	69	1	4
6525	-10	90	3	3
6471	-16	117	2	4
7106	-6	76	1	5
6898	-20	112	1.5	5
1324	-13	121	1.6	2.5
2683	-10	78	1.5	3.2
6556	-20	122	2	2.5
5562	-32	122	2	2.5
8518	-12	98	3	5
6435	-9	68	2.5	4
7057	-11	99	2.5	5
6308	-8	67	2	3
6325	-23	98	2	2.5
6535	-13	99	2	3
6831	15	-18	1.5	3.5
6935	-9	108	2	2.5
7104	-13	92	2	2.5
5023	0	60	1.5	3
6233	-1	62	2	3
4476	2	43	2	3
4501	-9	73	2	2
7191	-31	118	3	2.5
1355	-21	122	2.5	4
1396	-19	105	3	2.5

* Among the cases with electrocardiograms showing high P waves these had been diagnosed as pure mitral stenosis.

Summary Total cases, forty three. Thirty eight had an amplitude of P₁ greater than 1 mm (88 per cent), thirty-eight had an amplitude of P₂ greater than 3 mm (88 per cent), and twenty seven had an amplitude of P₂ equal to or greater than 3 mm (63 per cent). Indexes varied from +15 to -41 (four had positive values). Fourteen had indexes of -15 or below (33 per cent), twenty five had indexes of -10 or below (58 per cent), and twenty five had angles of +90 degrees or more (58 per cent).

TABLE 3—*Mitral Stenosis and Regurgitation*

Plate No	Index, Mm	Angle, Degrees	P ₁ , Mm	P ₂ , Mm
2173	-25	102	3	6
547	-25	127	2	5
948	-12	140	2	4
21	-22.5	96	2.5	5
332	-28.5	114	1	3
987	-18	112	1.5	2.1
1383	-25	107	1	3
1742	-14.5	88	1.5	2
2475	-32	109	3	2.5
3197	-6	85	1.5	1.3
4355	-21	132	2	2.5
4103	-3	68	2	3
50	-25	90	1.5	3.5
6689	-11	71	4	7

Summary Total cases, fourteen Twelve had an amplitude of P₁ greater than 1 mm (85 per cent), twelve had an amplitude of P₂ greater than 2 mm (85 per cent), and nine had an amplitude of P₂ equal to or greater than 3 mm (64 per cent) Nine had indexes below -15, twelve had indexes of -10 or below (85 per cent), and ten had angles equal to or greater than +90 degrees (71 per cent)

TABLE 4—*Mitral Stenosis and Aortic Regurgitation*

Plate No	Index, Mm	Angle, Degrees	P ₁ , Mm	P ₂ , Mm
270	-15	90	1.8	2.5
4170	-12	73	3.5	5.5
7182	10	-47	1	3
7786	5	53	1.5	3
8017	-10	90	1.5	3.5
2189	-11.5	76	1.3	3.3
4171	2	58	4	3
2419	19	-4	1.2	1.5
3626	-9	72	1	3
6647	42	-32	2	4

Summary Total cases, ten Eight had an amplitude of P₁ over 1 mm (80 per cent), nine had an amplitude of P₂ over 2 mm, and eight had an amplitude of P₂ of 3 mm or over (80 per cent) Two had an angle of only 90 degrees or over Four cases only had indexes of -10 or below (40 per cent), and one case only had an index of -15

TABLE 5—*Mitral Stenosis and Regurgitation and Aortic Stenosis and Regurgitation*

Plate No	Index, Mm	Angle, Degrees	P ₁ , Mm	P ₂ , Mm
7682	-8	75	1.5	4
7721	-17	100	2	4.5
3974	0	62	3	5
3983	0.5	60	3	3
6800	-26	110	1.5	3.5

Summary Total cases, five All had amplitude of P₁ greater than 1 mm, all had amplitude of P₂ of 3 mm or over All had angles of 60 degrees or over, two had angles of 90 degrees or over (40 per cent), and two had indexes of -10 or below

Summary of Tables 3, 4 and 5 Total cases, twenty-nine Twenty-five had amplitude of P₁ greater than 1 mm (86 per cent), twenty-six had amplitude of P₂ equal to or over 2 mm, and twenty-two had amplitude of P₂ equal to or over 3 mm (76 per cent) Eighteen had indexes of -10 or below Twenty-four had angles of 60 degrees or more, and fourteen had angles of 90 degrees or more (48 per cent)

TABLE 6—Miscellaneous Conditions

Plate No	Index, Mm	Angle, Degrees	P ₁ , Mm	P ₂ , Mm	Clinical Diagnoses
4166	10	48	3	2	Arteriosclerosis, cerebral embolism, sino auricular tachycardia
6148	—17	170	2	4	Effort syndrome, sino auricular tachycardia
2057	—10	72	1	3	Arteriosclerosis, sino auricular tachycardia
928	—15	123	1.5	2	Cardiac neurosis, sino auricular tachycardia
809	0.5	61	1.6	5	Sino auricular tachycardia
1313	—13	78	0.5	3	Pericarditis (?), mitral stenosis (?), sino auricular tachycardia
1256	1	57	1.5	5	Goiter, toxemia of pregnancy, sino auricular tachycardia
908	3	56	2.1	5	Nephritis, hypertension, arteriosclerosis
3295	—0.5	65	1.5	2	Nephritis, hypertension, arteriosclerosis, sino auricular tachycardia
4358	5	83	2.5	2.5	Nephritis, hypertension, sino auricular tachycardia
6410	19	23	2	4	Hypertension, congestive heart failure
6476	31	4	2.5	4	Hypertension, congestive heart failure, sino auricular tachycardia
6601	1.5	59	1	6	Exophthalmic goiter, sino auricular tachycardia
6716	0	54	1.5	4	Coronary sclerosis, sino auricular tachycardia
6771	—27	110	1	4.5	Sino auricular tachycardia
7001	14	5	2	1.5	Nephritis, hypertension, coronary sclerosis
7031	22	7	2	4	Hypertension, cardiac hypertrophy, arteriosclerosis
7042	45	35	1.5	3	Hypertension, arteriosclerosis, sino auricular tachycardia
7162	—1	63	1	4.5	Essential hypertension
8281	19	30	2.1	4	Hypertension, arteriosclerosis, hemiplegia
7156	10	30	1	5	No disease
6575	—36	128	2	4	Mitral stenosis (?), sino-auricular tachycardia
6588	—11	73	1	4.6	Mitral stenosis (?), sino auricular tachycardia
7063	9	28	1.5	2.5	Rheumatic history, normal heart
2954	—35	85	2	1	Arteriosclerotic and syphilitic heart, sino auricular tachycardia
4654	—7	58	1.5	2	Perinephritic abscess, sino auricular tachycardia
3854	—7	94	1	4	Hypertension, varicocele
5480	15	10	2	3.2	Chorea (?), mitral stenosis (?), sino-auricular tachycardia
3730	2	35	3	3	Nephritis, hypertension
1445	—8	75	2	3	Phthisis (?), mitral stenosis (?)
6820	—7	78	1	4	Mitral stenosis (?), sino auricular tachycardia
6857	—8	100	2	2	Hypertension, uremia, prostatitis
6487	6	46	1	3	Nephritis, hypertension, sino-auricular tachycardia
7068	—2	65	2	4	Congenital heart (?), ventricular septal defect, sino auricular tachycardia
7654	8	59	2	6.5	Congenital heart, septal defect, heart block
6590	—28	118	2	4	Congenital heart, septal defect, patent ductus arteriosus, sino-auricular tachycardia, pulmonic stenosis (?)
7170	—12	110	2	1	Congenital heart, ventricular septal defect (?), pulmonic stenosis (?), sino auricular tachycardia
6459	—20	86	2.5	4	Congenital heart, pulmonic stenosis (?), sino auricular tachycardia
8121	2	57	2	3.5	Nephritis, hypertension, uremia
8155	5	53	1.5	5	Nephritis, hypertension, uremia, sino auricular tachycardia
8975	0	60	2.5	3	Sino auricular tachycardia, curvature of spine

* All cases having other than definite pulmonic stenosis or mitral stenosis encountered in the review of cases with electrocardiograms showing high P waves

Summary Total cases, forty-one, doubtful mitral stenosis, six, doubtful pulmonic stenosis, three, probable interventricular septal defect without pulmonic stenosis, two, total congenital heart cases, five. Sino auricular tachycardia (heart rate of 100 or more) without question of mitral stenosis or congenital heart defects, eighteen. Hypertension with or without nephritis, not included in foregoing listing, ten. Rheumatic history without evidence of heart disease, one, and no disease, one.

approximation was shown by the percentage of right axis deviation, in that 60 per cent of all cases had indexes equal to or below -10 , and 54 per cent had angles equal to or greater than 90 degrees

A further interesting and expected finding in this series of combined mitral lesions (Tables 3, 4 and 5) is that the mechanism involved causes an increase in the amplitude of P but a decrease in the negativity of the indexes and in the positive angles (that is a lessened tendency to right axis deviation), as compared with the pure mitral stenosis cases

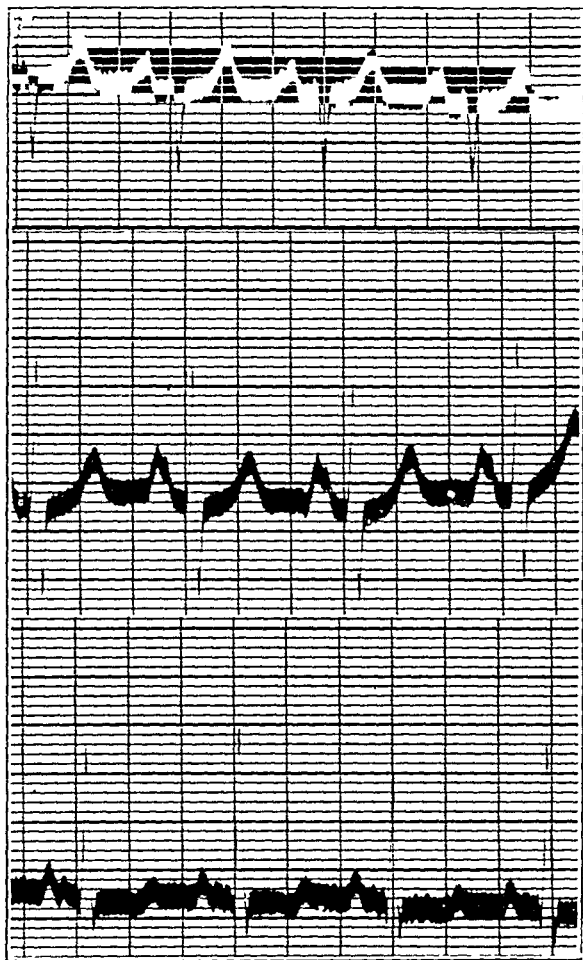


Fig 2—Leads I, II and III in well marked mitral stenosis, large P waves in Leads I and II and pronounced right axis deviation (right ventricular preponderance)

It is of much interest to note that twenty-nine of these forty-one miscellaneous cases (Table 6) showed conditions that could easily explain the abnormally high P waves. Mitral stenosis and pulmonic stenosis were probably present in the cases listed doubtful though the signs were not well enough marked to make a positive diagnosis. There were two other cases of congenital heart disease showing large P waves probably with interventricular defects, it is likely that the auri-

cles as well as the ventricles in some patients of this type are overworked with resultant hypertrophy or increased activity. Sino-auricular tachycardia is usually the result of sympathetic nerve stimulation, and it is a well recognized fact physiologically that such stimulation may result in increase in amplitude of the auricular deflection (and also of the T wave) of the electrocardiogram. The frequent finding of hypertension in some of the cases with high P waves may be significant—here again there is a possibility that the auricles as well as the ventricles

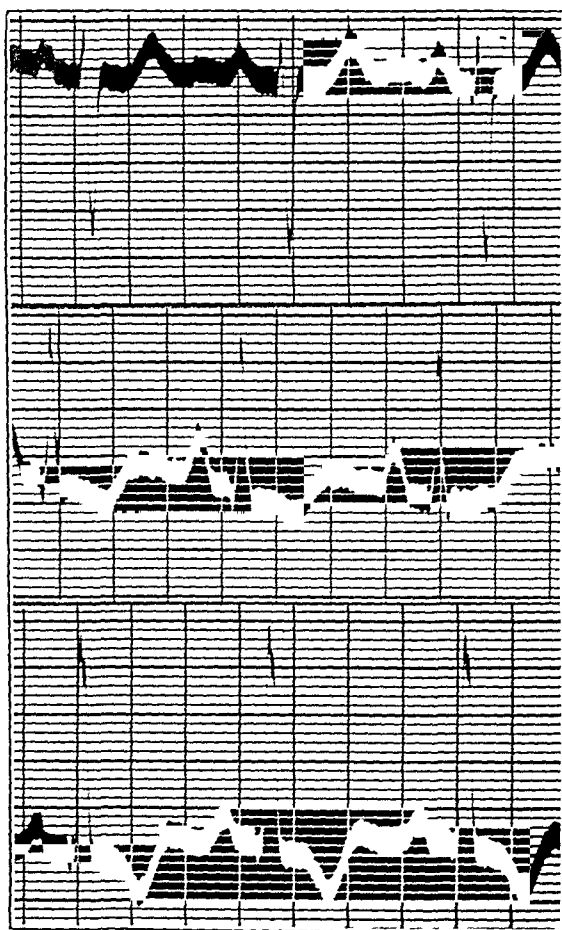


Fig 3—Leads I, II and III in congenital pulmonic stenosis, large P waves in Leads I and II and marked right axis deviation

may be overworked with resultant evidence of this increased activity, although large P waves are not the usual finding in hypertension. Only two of the whole group of the forty-one miscellaneous cases, and in fact only two of the entire series of 132 cases showing high P waves, remain unexplained in the presence of definite or doubtful mitral stenosis, definite or doubtful pulmonic stenosis, other congenital heart defects, sino-auricular tachycardia, or the possible influence of hypertension.

PART II

When we came to undertake the second part of the study mentioned above, that is, an analysis of the P waves as to their amplitude and of the angles and indexes in all cases of definite pulmonic stenosis in which electrocardiograms were made at the Massachusetts General Hospital since 1914, we discovered, as has previously been mentioned, that as a result of their increase in the amplitude of P they were all covered by Table 1, and had therefore all been analyzed. Seventy-nine per cent of all these cases showed an amplitude of P_2 equal to or greater than 3 mm. Seventy-nine per cent had an amplitude of P_1 greater than 1 mm, and 100 per cent had definite right axis deviation with angles of over 90 degrees and indexes of -10 or below. All cases had either P_1 over 1 mm or P_2 over or equal to 3 mm.

PART III

Turning to the third study mentioned we examined the plates of 100 unselected cases that had been diagnosed clinically as pure mitral stenosis (Table 7).

It is evident from Table 7 that in this series, 45 per cent of those showing P waves (that is, without fibrillation) had an amplitude of P_2 equal to or greater than 3 mm, and 78 per cent had an amplitude of P_1 greater than 1 mm, whereas 84 per cent had P_1 or P_2 equaling these high figures. Furthermore, one half of these cases showed the angles and indexes of right axis deviation. With regard to the angles in the entire series (with or without fibrillation) they were found to equal or exceed 90 degrees in 47 per cent of all cases, while the indexes in the total series were equal to or less than -10 in 52 per cent of the cases.

SUMMARY AND CONCLUSIONS

1. A study has been made of all the patients (132) who have shown by electrocardiogram during nine and one-half years at the Massachusetts General Hospital auricular or P waves greater than 0.1 millivolt (1 mm) in amplitude in Lead I or equal to or greater than 0.3 millivolt (3 mm) in Lead II. Of these cases nineteen (14 per cent) had definite congenital pulmonic stenosis, three (2 per cent) had doubtful congenital pulmonic stenosis, seventy-two (55 per cent) had definite mitral stenosis, six (5 per cent) had doubtful mitral stenosis, two (2 per cent) had probable interventricular septal defect, and eighteen (14 per cent) had sino-auricular tachycardia without question of mitral stenosis or congenital heart defects—a total of 120, or 91 per cent of the 132 cases. Only twelve cases (9 per cent) are not readily accounted for. Ten of these (8 per cent) had hypertension with or without nephritis, not included in previous groups.

TABLE 7—*Mitral Stenosis (Pune) **

Plate No	Index, Mm	Angle, Degrees	P ₁ , Mm	P ₂ , Mm
905	-15	120	3	3
1917	-11	69	2	2
2209	-17	102	2	2
2275	-15	130	1 8	2
1605	-8 5	81	1 5	2 7
3095	-13	95	1	4
3252	-19 5	120	2 2	2 2
2362	2	56	1 5	3 5
6653	-1	59	2	3
6685	-20	90	1	3
6675	-1	62	2	3 5
6801	-7	75	1 5	3 5
6865	-6	90	2 2	1 8
6939	-10	72	1 5	3
6948	5 5	25	2	4
8518	-12	98	2	5
7151	-5	69	1	4
6471	-16	117	2	4
7106	-6	76	1	5
6898	-20	112	1 5	5
1324	-13	121	1 6	2 5
2683	-10	78	1 5	3 2
6556	-20	122	2	2 5
1355	-21	123	2 5	4
7859	-3	64	Auricular fibrillation	
6620	-14	126	Auricular fibrillation	
1396	-19	105	3	2 5
6763	-10	133	Auricular fibrillation	
6064	-8	90	Auricular fibrillation	
6765	0	—	1 5	2
6818	-9	140	1 5	1 2
6884	-17	98	Auricular fibrillation	
6890	-2	70	Auricular fibrillation	
6906	-20	112	Auricular fibrillation	
7166	4	-35	Auricular fibrillation	
7169	10	10	1	2
6411	-17	90	Auricular fibrillation	
6430	-20	97	Auricular fibrillation	
6440	-11	127	Auricular fibrillation	
6945	-30	90	1	1
5311	-19	88	Auricular fibrillation	
6879	-23	102	1 2	2 5
6450	-24	121	Auricular fibrillation	
5608	-14	80	Auricular fibrillation	
5822	5	52	Auricular fibrillation	
5903	4	0	Auricular fibrillation	
3863	-20	87	Auricular fibrillation	
5119	-10	90	Auricular fibrillation	
5907A	-15	97	Auricular fibrillation	
5275	-15	109	Auricular fibrillation	
4685	0	58	Auricular fibrillation	
5545	-8	108	Auricular fibrillation	
6863	-5	55	Auricular fibrillation	
5792	4	40	Auricular fibrillation	
5907	-18	110	Auricular fibrillation	
3640	-15	115	Auricular fibrillation	
5849	-10	158	Auricular fibrillation	
6031	8	-15	Auricular fibrillation	
4183	2	-32	Auricular fibrillation	
4531	-16	115	Auricular fibrillation	
4833	-2	60	Auricular fibrillation	
4063	-11	60	Auricular fibrillation	
4376	4	-11	1	2
4200	-8	76	Auricular fibrillation	
4529	-2	68	Auricular fibrillation	
3780	-12	115	Auricular fibrillation	
4717	12	7	Auricular fibrillation	
4685	2	55	Auricular fibrillation	
4710	5	-17	1	2
3608	16	5	1	2
3716	3	46	1	2
5562	-32	122	2	2 5
8518	-12	98	3	5
6435	-9	68	2 5	4
6064	-8	90	Auricular fibrillation	
7057	-11	99	2 5	5
6308	-3	67	2	3

TABLE 7—*Mitral Stenosis (Pure) *—Continued*

Plate No	Index, Mm	Angle, Degrees	P ₁ , Mm	P ₂ , Mm
6325	-23	98	2	2.5
6535	-13	99	2	3
6831	15	-18	1.5	3+
6774	-44	161	2	2
6935	-9	108	2	2.5
7101	-13	92	2	2.5
5023	0	60	1.5	2
6233	-1	62	2	3
4476	2	42	2	3
4501	-9	73	2	2
6525	-10	90	3	3
2159	8	0	Auricular fibrillation	
4491	5	47	Auricular fibrillation	
8160	-5	74	Auricular fibrillation	
2450	-16	82	Auricular fibrillation	
3339	-18	142	2	2
3370	9	-16	1	1
2303	4	52	1.5	2.2
3182	-10	80	1.2	1
1742	-15	86	1.5	2.5
3319	1	57	1	1
3252	-20	120	2	2.5
3287	-6	69	1	1.5

* All were patients in the Massachusetts General Hospital electrocardiographed from 1918 to January, 1921.

Summary Total cases, 100, including forty-two cases of auricular fibrillation. Of the remaining fifty-eight, forty-five had P₁ greater than 1 mm amplitude (78 per cent) and thirty-nine had P₂ greater than 2 mm. Twenty-six had P₂ equal to or greater than 3 mm (45 per cent), forty-nine had an abnormally large P₁ or P₂ (81 per cent). Of the total (100) cases, forty-seven had angles equal to or greater than +90 degrees, seventy-four had angles equal to or greater than +60 degrees. Fifty-two had indexes equal to or below -10, thirty-one had indexes equal to or below -15. Of the fifty-eight cases with normal rhythm the indexes and angles were as follows: twenty-six had indexes equal to or below -10, sixteen had indexes equal to or below -15, forty-three had angles equal to or greater than 60 degrees, and twenty-seven had angles equal to or greater than 90 degrees.

2 Of all the nineteen patients with congenital pulmonic stenosis electrocardiographed at the Massachusetts General Hospital from 1914 to 1924 (nine and one-half years) 79 per cent had an abnormally large P wave in Lead I, greater than 1 mm in amplitude, 84 per cent had an abnormally large P wave in Lead II, equal to or greater than 3 mm in amplitude, 68 per cent had both, and 100 per cent had one or the other. Also, all showed abnormal right axis deviation (right ventricular preponderance).

3 Of the 100 unselected consecutive patients with mitral stenosis electrocardiographed at the Massachusetts General Hospital fifty-eight had normal rhythm. Forty-two showed auricular fibrillation. Of the fifty-eight cases with normal rhythm forty-nine (84 per cent) showed either an abnormally large P₁, P₂ or both.

4 An amplitude of P₁ greater than 1 mm, of P₂ equal to or greater than 3 mm, an electrical axis of 90 degrees or more, and an axis index of -10 or less are consistently found in congenital pulmonic stenosis.

5 A P₁ consistently greater in amplitude than 1 mm or a P₂ equal to or greater than 3 mm points to the possibility of an existent mitral stenosis, if both of these conditions are present there is greater likelihood of the condition; and if either of these findings is accompanied by right axis deviation, in the absence of pulmonic stenosis, the diagnosis of mitral stenosis is probable.

THE ACTION OF PARAPHENYLENDIAMIN

AN EXPERIMENTAL STUDY

KAETHE W DEWEY, M D

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In the course of studies on the lymphatic system of the eye I made use of a drug that is known to produce edema of the orbital tissue and bulging of the eyeball. This drug is paraphenyldiamin hydrochlorid, which is used in dyeing furs and feathers and which also is the chief constituent of certain hair dyes. According to Birch-Hirschfeld,¹ the edema produced by this substance is essentially stasis of lymph, the result of which is dilatation of the lymph vessels, and he states that he has been able by this means to demonstrate a lymphatic apparatus in the orbital tissue. My observations of the action of paraphenyldiamin on the lymphatic system have been discussed in a previous paper.² Because of the peculiar symptom complex that follows the injection of paraphenyldiamin and because of very marked differences in the effect of the drug in different species of animals, I carried on the experiments on a much larger scale than my original purpose called for. Many of the symptoms arising from paraphenyldiamin poisoning bear a peculiar resemblance to those observed in poisoning from a group of substances collectively called "histamins." The question of a possible relationship of paraphenyldiamin to the histamin group was, however, not included in my experimental study of the former drug.

To those familiar with the symptoms of histamin poisoning their similarity to the symptoms of paraphenyldiamin poisoning in men engaged in dyeing furs and feathers and occasionally in people using the hair dye must indeed be striking. Kobert³ states that in 30 per cent of the men who are employed in dyeing skins the effect of the drug is not only manifested by eczematous eruptions and other affections of the skin, but that the respiratory tract also is involved, and that periodical attacks of asthma occur. These attacks resemble those of genuine so-called bronchial asthma even in the composition of the sputum in which Charcot-Leyden crystals, Curschmann's spirals and Ehrlich's eosinophil cells are found.⁴ In view of these facts Erdmann,⁵ the

1 Birch-Hirschfeld, A. Die Krankheiten der Orbita, Graefe-Samisch, Leipzig, 9 112-114 (Part II) 1907

2 Dewey, K. W. A Study of the Lymphatic System of the Eye, Anat Rec 10 125, 1925

3 Kobert, P. Lehrbuch der Intoxikationen, Stuttgart, 1906, 2 653

4 Griegern. Ueber eine gewerbliche Vergiftung beobachtet bei der Rauchwarenfärbung mit Paraphenyldiaminpräparaten, welche unter dem klinischen Bilde eines Bronchialasthmas verläuft, Verhandl d Cong f inn Med 20 457, 1902

5 Erdmann, E. Ein neues Verfahren zum Färben von Pelzwerk, Ztschr f ang Chemie 8 424, 1895

inventor of this dye for skins and feathers, was induced to make the express statement that the drug is not applicable for the dyeing of human hair. The symptoms that may arise after the use of the drug as a hair dye are eczema, lacrimation, reddening and swelling of the conjunctiva, chemosis, glaucoma and exophthalmos, also such general symptoms as vomiting and diarrhea may be observed. In a case described by Puppe,⁶ skin eruptions appeared on the scalp and the neck the same day the woman applied the hair dye, the next day the face was so swollen that she could not open the eyes. All symptoms disappeared within a short time. Puppe was told by the patient that similar symptoms had occurred in other women who had used the dye. Pollack⁷ reported a case in which the symptoms appeared after the third application, several months had elapsed since the first and second applications. The symptoms were itching, burning and eczematous swelling of the skin, lacrimation, swelling of the lids and exophthalmos.

In animals the effects vary considerably with the species. It may be said that there is a marked idiosyncrasy for the drug. The observations with the hair dyes emphasize this, many people apparently have no symptoms from it while some react more or less violently.

Paraphenylenediamin is considered as being on the borderline of the blood poisons. If it is mixed with blood, brown discoloration and the formation of methemoglobin is observed. In the body brown discoloration also takes place, but methemoglobin is not formed, or is formed only to a slight degree. There is no explanation yet for the action of paraphenylenediamin and the peculiar changes about the eye. Puppe and also Kunkel⁸ are of the opinion that the blood and the blood vessels are essentially involved in the development of the intoxication edema which is the most characteristic feature of paraphenylenediamin poisoning. Tainter and Hanzlik,⁹ in testing the drug in rabbits, found that while specific edema of the head and neck develop in from one to three hours after subcutaneous injection of the drug (0.19 gm per kilogram) there is at the same time a relative increase in the hemoglobin and the total solids of the blood due to the escape of fluid from the circulation, this indicates that an increase in vascular permeability may be a factor in the production of the edema.

The features observed in paraphenylenediamin poisoning described in the foregoing vividly recall the peculiar symptoms characteristic of

⁶ Puppe. Ueber Paraphenylenediamin Vergiftung, *Vierteljahrsschr. f. gerichtl. Med.* **12** 1161 (Supp.) 1896, p. 1161, quoted by Matsumoto (Footnote 13).

⁷ Pollack, E. Ein Fall von Paraphenylenediaminvergiftung, *Wein. klin. Wchnschr.* **13** 712, 1900.

⁸ Kunkel, A. J. *Handbuch der Toxikologie*, Jena, 1901, p. 616.

⁹ Tainter, M. K., and Hanzlik, P. J. The Mechanism of Edema Production by Paraphenylenediamin, *Proc. Soc. Exper. Biol. & Med.* **20** 497, 1923.

histamin poisoning It would seem that these two substances are too widely different for a common action, yet we know today that a variety of substances that apparently are entirely unrelated in their chemical make-up have the typical histamin action First of all, it was found that the substances termed "histamin," "peristaltic hormone," "vasodilatin" and "motlin" are all the same substance It was shown that other substances, like pituitary extract, although from other tissue organs also have the typical histamin action, that in fact this action is common to a large group of poisonous substances of animal and vegetable origin With reference to the edema from histamin poisoning the view is expressed that it is due to the action of histamin as a poison to the capillary endothelium "This again," Abel and Kubota¹⁰ state, "brings them into relation with certain metallic poisons, such as arsenic and gold salts which were long ago classed by Huebner as 'capillary poisons,' and which when introduced in appropriate doses into the general circulation, produce a shocklike prostration" They remark further on

The existence of these points of community, in the action of substances so utterly unrelated chemically as histamin and certain metallic ions, forbids any assumption that the production of similar effects by unknown constituents of some organ or tissue indicates the presence therein of histamin itself, or of any substance chemically related to it

The dog is the animal most susceptible to the toxic action of paraphenylenediamin introduced subcutaneously The lethal dose is 0.1 gm per kilogram of body weight, with death occurring from two to three hours after the injection Half this dose is sufficient to produce marked symptoms In the rabbit very much larger doses are required and the injections may be repeated, but ultimately the same effect will be obtained Intravenous injections, according to Pollack, do not produce these characteristic symptoms However, he tested this only once My own repeated experiments showed that his statement is not correct, and Kobert and Dubois and Vignon¹¹ also state that they obtained edema of the subcutaneous tissue, and especially about the eye, by introducing the drug directly into the circulation The peculiar eye symptoms may also be produced by scarification of the skin, as in fact the effects of the hair dye on the skin would lead us to suppose

The symptoms of paraphenylenediamin poisoning that we observe in dogs have been described by Grunert as follows The earliest and most

10 Abel, J, and Kubota, S On the Presence of Histamin (β -Iminazolviethylamin) in the Hypophysis Cerebri and Other Tissues of the Body and Its Occurrence Among the Hydrolytic Decomposition Products of Proteins, *J Pharm & Exper Therap* **13** 243 (June) 1919

11 Dubois and Vignon Action physiologique de para et métaphénylène-diamine, *Compt rend Soc de biol* **107** 533, 1888

striking symptoms are chemosis and exophthalmos with an increase of the intra-ocular pressure. This is accompanied by increased lacrimal and mucous secretion of the conjunctivae and an increase of the saliva. With a large dose the edema of the orbit passes over to the face and to the neck. There is vomiting, diarrhea, paralysis, trembling, apathy, and under dyspnea and convulsions death occurs in coma within from three to six hours after the injection. Anatomically, the poisoned animals show serious effusion of the orbital cellular tissue, black discoloration of the lacrimal glands, slight brown discoloration of the tongue and gingivae, swelling of the entire mucosa of the upper air passages, and above all edema of the tongue and glottis. The other findings in the inner organs are in general negative. Edema of the glottis is to be considered as the cause of death. Besides the lacrimal gland, the gland of the nictitating membrane is discolored, some portions of the orbital gland also are sometimes slightly browned, both submaxillary glands are always, although slightly, discolored. On the other hand, the parotid, the thyroid and the thymus glands, the lung, liver, pancreas, testes and Peyer's plaques are always normal.

Grunert¹² does not explain where the fluid in the edematous tissue comes from. It is a fact that the edema fills the entire orbit as early as one hour after the injection and disappears again in one day without leaving any demonstrable traces. No cellular infiltration is ever found about the glands as an indication of an irritation. Grunert states that the edematous fluid pressed out of the tissue does not differ in reaction nor in its other chemical behavior from normal lymph, but is well distinguished from inflammatory edema. He believes, therefore, that we must assume that the substance is made ineffective or is bound in the lacrimal gland. Not so in the salivary glands, which excrete the poison unsplit. The orbital edema is therefore, he states, congestion or stasis of normal lymph, which, according to Matsumoto, is apparently in the state of coagulation, for it does not flow off when the edematous tissue is cut into. Puppe believes that it is a stasis edema, perhaps due to the formation of thrombi in the veins.

Matsumoto and Kunkel are of the opinion that the substance circulating in the blood passes from the peripheral capillaries into the lymph and there produces certain chemical changes of the lymph impeding its forward motion. An analogy in the action of this poison is offered by potassium iodid, which also produces coryza and edema of the glottis. Grunert asserts that he has shown that the lacrimal gland by a special property of the protoplasm of its secreting cells is able to render this poison chemically ineffective. Tainter and Hanzlik found that localiza-

¹² Grunert, K. Die Augensymptome bei Vergiftung mit Paraphenylendiamin nebst Bemerkungen über die Histologie der Tranendrüse, *Ber u d. Versamm d ophth Gesellsch*, 1903, pp 31-32, 208.

tion of the edema in the head and neck of the rabbit is not connected with an increased concentration of paraphenyldiamin in these regions, since quantitative estimations of the paraphenyldiamin in the saliva and in the edematous fluid showed the concentration to be less or no greater than that in the blood plasma. They also state that oxidation products are not concerned in the edema.

Matsumoto¹³ tested the drug on frogs and mice as well as on dogs and rabbits. If frogs are brushed with a 15 per cent solution of paraphenyldiamin on different parts of the body, torpor, paresis and gradually complete paralysis are observed. At the necropsy the superficial muscles are markedly blue, this coloration increases in contact with the air. Matsumoto believes that the oxygen of the air diffuses through the skin to the muscles and that oxidation does not take place through the oxygen circulating in the blood. Subcutaneous injection of 0.01-0.02 gm of paraphenyldiamin kills frogs weighing 40-60 gm. Paresis and paralysis are the chief symptoms. The subcutaneous cellular tissue contains large quantities of clear fluid. There is pigmentation of the liver and spleen and bluish red coloration of the superficial muscles. Mice are killed by as little as 0.005 gm of paraphenyldiamin. Paresis is soon observed and death occurs under convulsions within one hour. There is no edema of the subcutaneous tissue nor pigmentation of the liver and spleen worth mentioning. The muscles turn brownish red. Rabbits may be killed by intravenous injection of 0.2 gm of paraphenyldiamin per kilogram of body weight. The symptoms are salivation, edema of the tongue, trismus, subcutaneous edema of the lower portion of the face and the upper portion of the neck, paresis, convulsions, dyspnea, and even suffocation followed by death. The eye symptoms are not prominent, however, exophthalmos, chemosis and edema of the lids are sometimes observed. In dogs he observed the same symptoms as other authors. To him the most plausible explanation for the phenomenon seemed that the paraphenyldiamin circulating in the blood is in part eliminated into the lymph from the peripheral capillaries. About the head the poison meets the proper conditions for certain chemical alterations which lead to coagulation or at least impeded flow of the lymph. The edema in these conditions differs from that in man, Matsumoto believes, when cutting into such tissue, this appears gelatinous, while in edema in man clear fluid flows out.

A summary of my experiments will show that, in general, the symptoms I observed are the same as those reported by the writers

¹³ Matsumoto, H. Ueber die Giftwirkung des Paraphenyldiamins, I. D., Würzburg, 1901.

I have mentioned, but that there are some observations which have not been reported or duly emphasized by these writers

The most characteristic effect of paraphenylenediamin, namely, edema of the orbital tissue with exophthalmos and lacrimation, is observed best in the dog. This animal is the most susceptible to the action of the drug. The lethal dose is 0.1 gm subcutaneously, with death occurring from two to three hours after the injection. Rabbits can stand very much larger doses than dogs. The dose of 0.1 gm per kilogram, fatal to dogs, has to be given several times to rabbits before the characteristic symptoms arise. A single dose of 0.5 gm will produce death within from two to three hours. Edema of the glottis is the immediate cause of death. Exophthalmos is rarely observed in rabbits. Edema of the subcutaneous tissue of the face and neck, of the glottis and the upper air passages is the most conspicuous symptom. In frogs the characteristic symptoms are of a nervous nature: torpor, paresis and paralysis. In guinea-pigs they are exclusively nervous: excitement and convulsions. There is no swelling of the subcutaneous tissue. The symptoms in dogs are restlessness very soon after the injection, vomiting, purging, swelling of the conjunctivae and the lids, well noticeable one and one-half hours after the injection, sensitiveness of the eyes to light, uneasiness, groaning, respiratory disturbances, and a comatose condition after three and one-half hours when the dose is less than 0.1 gm per kilogram. Cats react very similarly to the drug. Constriction of the pupil, respiratory disturbances and edema of the glottis are conspicuous symptoms.

Since the statements about the effects of paraphenylenediamin in rabbits vary somewhat and apparently the experiments with them by other writers have not been extensive, I give a table of my observations on rabbits. A table of the effect on guinea-pigs is added, evidently no tests on those animals have yet been made.

When we consider these symptoms, the action of paraphenylenediamin seems to differ widely in animals. The strictly localized edema, the most conspicuous symptom in dogs, cats and rabbits, seems to have nothing in common with the nervous manifestations, the main symptom observed in guinea-pigs, frogs and mice in which edema does not occur. The peculiar localization of the edema is such a striking feature in the former group of animals that it has received the chief attention from all investigators. No satisfactory explanation of this singular action of paraphenylenediamin has yet been offered. Some writers have attempted to explain the steps in which the edema may be produced, it has not been suggested that primarily the cause may be from the nervous system. Nervous manifestations, however, are actually observed in dogs and cats very early, they are even the very first symptoms. Soon

TABLE 1—Effect of Paraphenylenediamine on Rabbits

Rabbit No.	Weight, Gm	No of Injections	Dose per Kg of Body Weight, Gm	Kind of Injection	Interval Between Injections	Interval Between First Injection and Death	Time of Onset of Symptoms	Symptoms	Cause of Death	Remarks
1	1,080	3	0.1	Subcutaneous	24 hours	4 days	From 5 to 6 hours after first and second injections, 2 hours after third injection, later 12 hours	Edema on face and neck, salivation, torpor, swelling always very much reduced after 24 hours	Not edema of the glottis, intoxication?	Apparently this rabbit was more susceptible to the drug than most rabbits
2	1,580	5	0.1	Subcutaneous	24 hours between third and fourth injections, 48 hours between fourth and fifth injections	5 days	No symptoms after the first and second injections, swelling, torpor, anorexia 2 hours after third injection, no edema after fourth and fifth injections, torpor and anorexia remained	Edema, salivation, torpor, anorexia, edema following the third injection did not arise after the fourth and fifth injections, torpor and anorexia remained	Not edema of the glottis, intoxication?	
3	800	3	0.15	First, intravenous, second and third, subcutaneous	19 hours between first and second injections, 2 hours between second and third injections	23 hours	No symptoms after the first and second injections, they arose after third injection	Edema on face and neck, salivation, dyspnea after the third injection, death 2 hours after the third injection	Edema of the glottis	Violent local reaction, sloughing of epidermis at place of injection
4	1,645	3	0.15	First and second subcutaneous, third intravenous	4½ hours between first and second injections, 20 hours between second and third injections	25 hours	No symptoms after the first injection, they arose late after the second death ½ hour after third injection	Slight edema, torpor and anorexia after the second injection, death ½ hour after the third injection	Edema of the glottis	
5	510	1	0.3	Subcutaneous		12 hours	Several hours after the injection	Marked edema of the face, salivation, dyspnea	Edema of the glottis	Brown discoloration of muscles, no edema about eyes
6	1,070	2	0.35	Subcutaneous	18 hours	20 hours	No symptoms after the first injection, death 2 hours after second injection	Salivation, dyspnea, edema over lower portion of neck	Edema of the glottis	
7	985	1	0.3	Intravenous		16 hours	Symptoms several hours after injection	Enormous swelling of subcutaneous tissue over upper portion of neck	Killed before it had time to die from edema of the glottis	No edema about the eyes
8	780	1	0.5	Subcutaneous		2 hours	1½ hours after the injection	Dyspnea from edema of the throat and tongue	Edema of the glottis	Lockjaw but not from drug, several normal rabbits had it
9	1,400	4	0.1	Intravenous	3 days	13 days (killed)		No symptoms		Marked sloughing of skin at place of injection
10	1,270	5	0.1	Subcutaneous	3 to 4 days	21 days		No symptoms		

TABLE 2—Effect of Paraphenyldiamin on Guinea-Pigs

Guinea-Pig Number	Weight, Gm	Number of Injections	Dose per Kg of Body Weight, Gm	Kind of Injection	Interval Between Injections	Interval Between Injection and Death	Time of Onset of Symptoms	Symptoms	Remarks
1	280	4	0.125	Subcutaneous	4 days	13 days	Early after fourth injection, death 50 minutes after the third injection	Eventuality	
2	280	3	0.125 0.250	Subcutaneous	4 days	9 days	Early after third injection, death 2 hours after the third injection	Convulsions	Sores at place of injections
3	480	2	0.140	Intraperitoneal	3 days	3 days	Symptoms arise slowly after the second injection, death over night	Twitching in hind legs, excitability, final symptoms could not be observed	
4	380	2	0.200	Intraperitoneal	3 days	3 days	One hour after second injection, death over night	Twitching in hind legs, excitability, final symptoms could not be observed	
5	600	2	0.058 0.150	Subcutaneous	3 days	3 days	Slight nervous manifestations after first injection, violent symptoms arose almost at once after second injection, death 35 minutes after injection	Convulsions until death	Guinea pigs 5 and 6 had been treated like frogs for 24 days by daily repeated external applications of the drug, no symptoms occurred, the animals gained in weight, but apparently there was some absorption
6	400	2	0.062 0.200	Subcutaneous	3 days	3 days	Slight nervous manifestations after first injection, violent symptoms $1\frac{1}{2}$ hour after the second injection, death 4 hours after injection	Animal constantly moves in a circle from right to left	

after the injection the animal becomes restless, there are disturbances in the locomotion, the head is thrown back, as in opisthotonos, before there are manifest indications of respiratory interference from bronchial spasm or edema in the air passages. Dubois and Vignon¹¹ also report such symptoms. It would not seem unreasonable to assume that the edema occurring in man and dogs is due to paralysis or irritation of the vasomotor nerves, resulting in an increase of the vascular secretion.

It is of interest to note that the manifestations of the toxic action of paraphenyldiamin in men employed in dyeing skins are of a type that may be classed with the so-called neuropathic affections, namely, certain skin eruptions which, like urticaria, herpes zoster, etc., are probably intoxication symptoms associated with nervous disturbances in the functions of the capillaries. Moreover, the bronchial asthma that attacks a rather high percentage of the men who are exposed to the action of paraphenyldiamin in their occupation is a "neurosis." The action of this drug may be in all cases on the nervous system, and nervous influences exerted on the capillaries may be the direct cause of the edema. The localization is a peculiar feature. The edematous swelling of the orbital tissue in dogs is very striking, but the edema in the upper air passages leading to death from suffocation is not less remarkable. In rabbits the edema of the subcutaneous tissue of the face and the neck, of the tongue and the throat is much more marked than about the eyes.

Why the action of a toxic substance on the nervous system should evoke a localized edema is not at once clear. We may assume that nervous influences may be exerted directly on the capillaries and that in the region of the eyes, in the throat, the face and the neck the capillaries may have certain properties peculiar to them and adjusted to functional activities in these regions, and that it is because of these properties that edema is produced by neuropathic influences in these regions only and not in others. This inference is possible in view of the fact that the transudates differ one from another in various localities. I refer here only to statements by Ziegler¹⁴ that the albumin content of pure transudates is not the same in all body cavities and tissues, but differs in a pronounced degree. The albumin content of transudations of the pleura, according to Reuss, is 22.5 per thousand, that of the pericardium 18.3, of the peritoneum 11.1, of the subcutaneous connective tissue 5.8, of the cavities of the brain and spinal cord 1.4. To these statements Ziegler adds the remark "These facts may be taken as a proof of the different constitution of the vessel walls in the various tissues of the body."

14 Ziegler, E. General Pathology, translation, New York, 1918, p. 153

For comparison let us recall some observations of the action of histamin in animals. Dale and Laidlow¹⁵ observed immediate vomiting and purging, profuse salivation and labored respiration followed by a period of collapse in a cat injected intravenously with 10 mg of the hydrochlorid of histamin. There is a marked idiosyncrasy in the species of animals, especially in rodents. In the latter, the effects of intravenous and subcutaneous injections are conspicuous features in histamin poisoning. Death is due to asphyxia from constriction of the bronchioles by spasm of the muscular coats, perhaps also from increased secretion. With smaller doses there is recovery of the symptoms, a second injection before the effects of the first have entirely subsided causes renewal of the symptoms in an accentuated form, death ensuing in a few seconds. Large doses produce convulsive and obstructed respiration. In cats there is not such a marked discrepancy between the effects of intravenous and subcutaneous injections. Salivation, lacrimation and increased bronchial secretion are observed. Constriction of the pupil, even in the dusk, is a notable feature.

Concerning the bronchial spasm, Dale and Laidlow remark that it is impossible to say whether histamin has a direct effect on the respiratory center. Barger and Dale¹⁶ state that histamin increases the flow of lymph from the thoracic duct. Interesting are the views of Keeton, Koch and Luckhardt¹⁷ concerning the observations made by Dale and Richards¹⁸.

Their observations indicate a remarkable vascular action by histamin. Apparently it acts upon two distinctly different anatomical structures in the vascular system. It causes a transient vasoconstriction in the arterioles and a more delayed and prolonged vasodilating action on the capillaries due to inhibition of their tone. The main action then consists in opening up new capillary areas together with permeation of the capillary wall by blood plasma—Dale and Richards' observations indicating a loss of plasma and an increase in concentration of red corpuscles in the blood, as a result of histamin, have also been observed by Simon.¹⁹

The striking dissimilarity in the action of paraphenylenediamin in different animals has an interesting analogy in one of the most charac-

15 Dale, H, and Laidlow, P. The Physiological Action of β -Iminazolyethylamine, *J Physiol* **41** 318, 1910, Further Observations on the Action of β -Iminazolyethylamine, *J Physiol* **43** 183, 1911-1912.

16 Barger, G, and Dale, H. β -Iminazolyethylamine, a Depressor Constituent of Intestinal Mucosa, *J Physiol* **41** 499, 1911-1912.

17 Keeton, R W, Koch, F C, and Luckhardt, A B. Gastrin Studies, III, The Response of the Stomach of Various Animals to Gastrin Bodies, *Am J Physiol* **51** 454 (April) 1920, IV, The Response of the Stomach Mucosa to Food and Gastrin Bodies as Influenced by Atropin, *ibid* **51** 469 (April) 1920.

18 Dale, H H, and Richards, A N. The Vasodilator Action of Histamin and of Some Other Substances, *J Physiol* **52** 110 (July) 1918.

19 Simon, L G. Sur Quelques Effects des Injections de Secretine, *J de physiol et de path gen* **9** 79, 1907.

teristic actions of epinephrin or pituitary extract (histamin), namely, that on the plain muscle of the uterus Keeton, Koch and Luckhardt, in reference to this, state

It is well known that the virgin or nonpregnant uterus of the rabbit, dog, ferret, monkey or man always contracts after adrenalin treatment whereas in the cat, guinea-pig and rat this may cause a relaxation, and that only the pregnant uterus is caused to contract

SUMMARY

1 There is a marked difference in the susceptibility to paraphenyldiamin not only in species of animals but also in individuals of the same species

2 In people with an idiosyncrasy for the drugs paraphenyldiamin (in an industrial occupation or after the use of the hair dye) produces local and general symptoms that resemble somewhat those observed in the dog The drug apparently is absorbed by the skin to some extent

3 Some animals (individuals, not species) show a more or less violent local reaction, resulting in sharp demarcation of an area with ultimate sloughing

4 Dogs are the most susceptible to the drug A fatal dose is 0.1 gm per kilogram of body weight with death occurring from two to three hours after the injection Marked symptoms are produced by 0.05 gm Cats react similarly

5 Doses fatal to dogs may be given from three to five times to rabbits without producing any symptoms if the intervals (from three to four days) are long enough to permit elimination of the poison With short intervals (from four to twenty-four hours) symptoms generally arise after the third injection A dose of 0.34 gm injected subcutaneously or intravenously produces death within twelve hours

6 Guinea-pigs, like rabbits, are not affected by a subcutaneous dose that is fatal to the dog Symptoms arise after the third or fourth injection of 0.125 gm, if the intervals are four days Intraperitoneal injections act more quickly If paraphenyldiamin is applied externally for some time, a small dose injected subcutaneously is sufficient to produce symptoms

7 Frogs are very susceptible to the poison applied externally, it is rapidly absorbed by the skin Death may occur one hour after the application

8 The symptoms in dogs, cats and rabbits are a localized edema In guinea-pigs, mice and frogs they are nervous manifestations paresis, paralysis or convulsions

9 The action of the drug is perhaps in all cases on the nervous system The edema about the head may be the result of vasodisturbances The localization may correspond to differences in the constitution of the vessel walls in the involved areas

INTERMITTENT AURICULAR FIBRILLATION

WITH FLEETING RAPIDLY RECURRING PAROXYSMS HAVING
IDENTICAL TYPE OF AURICULAR BEHAVIOR [†]

CHARLES C WOLFERTH, M D

PHILADELPHIA

It has become recognized during the last few years that paroxysmal auricular fibrillation is a relatively common disorder, although probably not so frequent as chronic fibrillation. According to the published descriptions of cases, it would appear that the paroxysms usually last hours or days, nevertheless, all clinicians of experience must occasionally have encountered paroxysms lasting only a few minutes, particularly in cases of hyperthyroidism but also occasionally in other forms of cardiac involvement.

Very brief paroxysms, not more than a few seconds in duration, have been very rarely observed. This may be due in part to the impossibility of recognizing the nature of such brief paroxysms by ordinary clinical methods and to the fact that unless they recur frequently they are recorded in electrocardiograms by mere chance. A careful search of the literature extending over the period of use of the electrocardiograph in clinical medicine for examples of such fleeting paroxysms reveals only four cases reported with electrocardiograms that leave no doubt as to the correctness of the interpretation. Possibly other cases not readily accessible have been overlooked.

Semerau,¹ in 1918, reported two cases in which fleeting paroxysms were recorded. In one of these, however, the paroxysms did not occur spontaneously but could be elicited by exertion or excitement. Smith and Moody,² in studying a patient who gave a history of paroxysmal cardiac attacks, were able to evoke short paroxysms of fibrillation during the expiratory phases of forced breathing. Recently, Wedd³ has reported a case in which coarse wave fibrillation was present when the patient entered the hospital, but on the following day many paroxysms of auricular fibrillation, some of which were extremely fleeting, occurred.

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1 Semerau, M. Ueber Rückbildung der Arrhythmia Perpetua, *Deutsch Arch f klin Med* **126** 161 (May 24) 1918.

2 Smith, F. M., and Moody, W. B. The Induction of Premature Contractions and Auricular Fibrillation by Forced Breathing, *Arch Int Med* **32** 192 (Aug) 1923.

3 Wedd, A. M. Clinical Auricular Flutter, *Ann Clin Med* **3** 69 (July) 1924.

In Wedd's patient, following the use of quinidin several fleeting paroxysms of auricular flutter also were recorded

A case we have recently studied is of interest on account of the remarkable brevity, general similarity to each other and rapid recurrence of paroxysms. A number were recorded sufficient to afford an opportunity of making observations on the mode of onset of fibrillation, comparisons of the course of the excitatory process in various paroxysms, the mode of offset and the duration of "postundulatory" pauses

The extreme brevity and rapid recurrence of paroxysms in the case to be reported, resembling the behavior of intermittent auricular flutter, which I have recently described,⁴ and intermittent paroxysmal auricular tachycardia, described by Kure and Sakai⁵ and others, has suggested that the name intermittent auricular fibrillation be reserved for this condition. The name has been used by some writers, rather unhappily, to apply to the ordinary clinical forms of paroxysmal auricular fibrillation

The patient was referred for electrocardiographic study by Dr Hobart A. Hare, to whom I am indebted for the privilege of reporting the case

REPORT OF CASE

T. S., a robust, healthy looking man, aged 56, had been seen by Dr. Hare from time to time during a number of years because of hypertension. No arrhythmia had been noted by Dr. Hare prior to June 11, 1924, when marked irregularity of the cardiac action was found. The examination was otherwise essentially negative except for marked diminution of the pulse in the right brachial, radial and carotid arteries. It was suspected that this was caused by some lesion of the aorta or innominate artery, possibly aneurysm, although no direct evidence of aneurysm could be obtained either clinically or by roentgen ray. There was no evidence of syphilis.

The electrocardiograms of June 11 are shown in Figures 1 and 2. Frequent premature auricular beats which occur irregularly are found. Some of the premature beats are isolated but others are followed by fibrillary after-effects of varying lengths, represented in the tracings by one up to ten irregular auricular oscillations and lasting from a fraction of a second to over two seconds. The majority of the after-effects are represented by four or five waves and have a duration of about one and one-half seconds. Their intermittent distribution and rapid recurrence are shown in the tracings. According to Dr. Hare's observation, this special form of arrhythmia must have lasted for a number of days. The lack of regularity in shape and time relations of the oscillations indicates that these disturbances of rhythm must be due to auricular fibrillation as they are far too irregular to be regarded as forms of so-called "impure" flutter.

The patient was sent back for another tracing June 21, and at this time there was found continuous fibrillation. Several tracings have been made since that

4 Wolferth, C. C. Intermittent (Impure) Auricular Flutter, with Special Reference to Onsets and Offsets of Paroxysms and the Effects of Vagus Stimulation, *Arch. Int. Med.* **35**: 42 (Jan.) 1925.

5 Kure, K., and Sakai, S. Ein Fall von intermittierend Auftreten der heterotoper Vorhofstachysystolie, *Deutsch. Arch. f. klin. Med.* **140**: 67 (July 25) 1922.

time, the last on Dec 4, 1924, all of which have shown continuous fibrillation. Cardiac compensation has been fairly well maintained in spite of several anginal attacks and the patient has carried on his usual work as a lawyer.

The continued repetition of fleeting paroxysms of auricular fibrillation observed during the first examination must be attributed to a peculiar functional state of the auricular muscle. The celerity with which the disturbances followed one another indicates that the muscle was in an advanced stage of what Garrey⁶ has called the "fibrillary state." Equally important for the production of such a form of arrhythmia was the fact that none of the disturbances were able to perpetuate themselves. Some time in the ten days intervening between

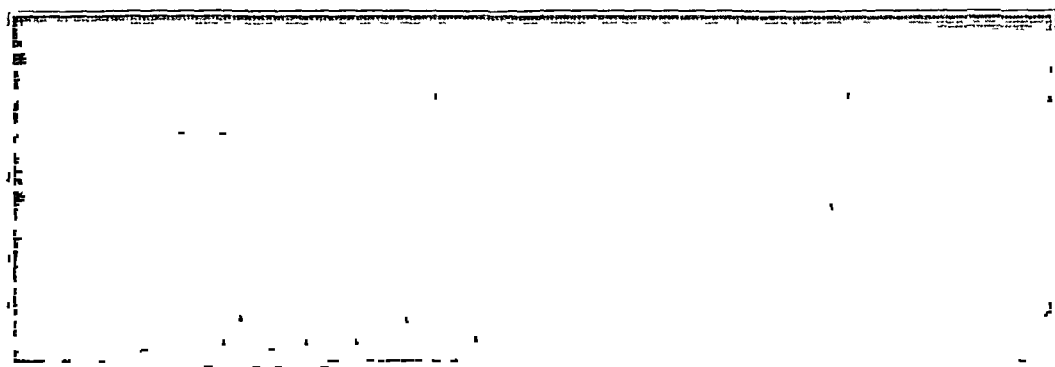


Fig 1—Several isolated premature auricular beats in Lead II, characteristic alternation of sinus rhythm and short paroxysms of auricular fibrillation, similarity of auricular portions of curves, a brief paroxysm in the left hand corner of bottom strip. Only those paroxysms ending alike (after the fifth auricular oscillation) have postundulatory pauses of similar length.

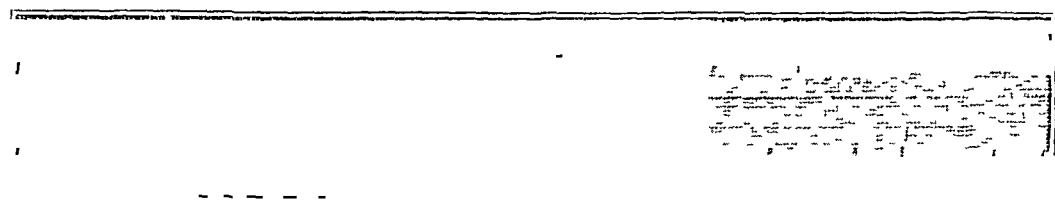


Fig 2—Isolated premature auricular beats in Lead III, one extremely brief paroxysm, one of intermediate length, and the longest one recorded.

the first and the second examination, a change must have occurred in the properties of the auricular muscle whereby it became possible finally for a paroxysm to be extended indefinitely into continuous or chronic fibrillation.

The course of events observed in this patient emphasizes sharply the fact that such alterations in functional properties of cardiac muscle, which make easy the spontaneous entrance of auricular fibrillation, are not exactly identical with the conditions that permit of the continuance

⁶ Garrey, W. E. Auricular Fibrillation, *Physiological Rev* 4 215 (April) 1924.

of fibrillation Normal heart muscle, if we may judge from experimental evidence, does not permit auricular fibrillation to perpetuate itself long, although there are wide individual variations in the lengths of paroxysms When heart muscle is altered to the fibrillary state, the form of arrhythmia (paroxysmal or chronic) will depend on either preservation or loss of ability to terminate fibrillation spontaneously Clinical observation appears to indicate that in many cases the first onset of fibrillation continues as chronic fibrillation In such cases what might be called the functional capacity of terminating fibrillation is lost coincidentally with, or possibly even before, the development of the fibrillary state

Whether or not in our case the remarkable brevity of paroxysms in spite of the highly fibrillary state indicated temporary preservation of a normal type of reaction or was due to some accidental disarrangement in the mechanism that ordinarily maintains fibrillation in certain damaged hearts, the data do not permit us to say In two cases of intermittent auricular flutter, I was able to present evidence in explanation of the brevity of paroxysms⁴ Although the mechanism differed somewhat in the two cases, the termination of flutter in both was due to failure of the excitatory process to elect a path in which equilibrium could be established between the rate of propagation of the impulse and the duration of the refractory phase Possibly an analogous explanation might account for the brevity of paroxysms in our present case, but the curves in fibrillation are so irregular as to make analysis impossible

MODE OF ONSET

Garrey⁶ states that auricular fibrillation may arise from a normal impulse from the sinus region or any other impulse, no matter where it originates In the clinical instances previously reported, nearly all onsets recorded were initiated through premature auricular beats I have recently called attention to a similar rôle of premature auricular beats in exciting paroxysms of auricular flutter⁴ That clinical fibrillation may take its origin directly from a sinus beat has been shown clearly in a tracing published by Semerau¹ In Semerau's case, fibrillation had been momentarily interrupted by vagus stimulation, and on its resumption was initiated by a sinus beat

In our case premature auricular beats were concerned in the onsets of all paroxysms recorded Except for the after-effect of fibrillation, they appeared identical in all respects with the numerous isolated premature beats They all bore a close enough relationship to preceding beats to suggest coupling, but the lengths of the periods varied from 0.4 to 0.46 second Whether these extrasystoles be regarded as the direct exciting cause of the fibrillation or as an intermediate bridge from the

preceding sinus beat depends on one's attitude toward the unproved view that extrasystoles are circus contractions. The point of practical importance is that auricular extrasystoles may and usually do play a highly conspicuous rôle in the initiation of auricular fibrillation provided the fibrillary state is present.

PATH OF THE EXCITATORY PROCESS

Examination of the auricular portion of the curve in any of the paroxysms recorded appears to indicate an irregular, haphazard course of the auricular excitatory process, manifested by markedly irregular spacing and lack of uniformity in shape of the waves. Such a curve cannot be interpreted otherwise than as auricular fibrillation. Nevertheless, if all the paroxysms recorded in Figure 1 are compared, it is found that there is a remarkable similarity among them. They show significant variation in lengths of paroxysms only. So far as each one continues, the apparently irregular auricular curves can be practically superimposed on one another. Measurements of intervals between summits of waves vary by only slight differences. For example, in Figure 1, Strips 1 and 2, measurements of successive intervals between the first six summits, beginning with the normal P waves, are 0.41, 0.26, 0.37, 0.18, 0.22 (total 1.44) seconds, 0.42, 0.26, 0.36, 0.20, 0.21 (total 1.45) seconds, 0.42, 0.26, 0.36, 0.19, 0.22 (total 1.45) seconds, and 0.43, 0.25, 0.36, 0.18, 0.22 (total 1.44) seconds, respectively. A similar relationship is also found among all the paroxysms recorded in Leads I and III. We are therefore justified in assuming that in spite of the apparent irregularity of the excitatory process, it does not wander haphazard but proceeds in a definite manner over a somewhat devious but identical course so far at least as these brief paroxysms can be compared.

Identity of auricular behavior among paroxysms of auricular fibrillation has not been demonstrated previously. Lewis, Drury and Ilescu,⁷ in discussing clinical fibrillation, have called attention, however, to the fact that there appears to be a definite channel for which the central excitation wave shows a predilection, that sometimes it is forced out of this channel, but that, if this happens, the wave soon returns to its old path, repeating its old course and in the original direction. Lewis has repeatedly championed the view that in clinical fibrillation there is a single central excitation wave circulating in the auricular muscle. Such precisely circumscribed movements as occurred in our case could scarcely be explained without assuming the presence of a single central excitation wave.

⁷ Lewis, T., Drury, A. N., and Ilescu, C. C. A Demonstration of Circus Movement in Clinical Fibrillation of the Auricles, *Heart* 8:379 (Aug.) 1921.

OFFSETS OF FIBRILLATION AND "POSTUNDULATORY" PAUSES

The offsets, as in other recorded offsets of auricular fibrillation, are abrupt. Each was followed by a postundulatory pause. This name was apparently coined by Gewin⁸ to designate the pause after cessation of experimental fibrillation before resumption of normal sinus beating. Such pauses had been noted following *delirium cordis* by Hoffa and Ludwig⁹ as early as 1850. Winterberg¹⁰ regarded them as analogous to the pauses after auricular extrasystoles. Semerau¹ mentions their occurrence in his cases of clinical fibrillation.

Garrey⁶ has recently stated that a fibrillary beat may be initiated from the normal pacemaker and the impulse shuttle back and forth through auricular muscle producing rapid oscillations which may stop before the next impulse arises so that the normal rhythm is not disturbed in the least and there is no postundulatory pause.

In the condition mentioned by Garrey, the fibrillary wave presumably does not return to the sinus node. In longer paroxysms of auricular fibrillation, the centrifugal wave doubtless reaches the pacemaker as well as all other parts of the auricles so that, following the last stimulation of the pacemaker in this manner, time approximate to that of a normal cycle is required to build up and discharge an impulse. In Figure 1 the time of a normal cycle is approximately 0.82 second, and measurements from summits of isolated premature beats to summits of the next normal P waves vary from 0.99 to 1.01 seconds, indicating the presence of postextrasystolic pauses. Unfortunately, the curves do not permit of accurate measurements of postundulatory pauses, because ending of fibrillation is not sharply enough defined, but in the three paroxysms in Lead I that end similarly measurements from the summits of the last fibrillary waves to the summits of the following P waves are 1, 1.03 and 1.01 seconds, respectively, these correspond closely in time with the postextrasystolic pauses. All other postundulatory pauses are apparently shorter, varying from 0.79 to 0.88 second. It is of interest that even the most abortive manifestations of fibrillation, having a duration only a fraction of the length of a normal cycle, are followed by postundulatory pauses (Fig. 2).

Our measurements appear to indicate a wide variability in duration of the postundulatory pause. The variation may be more apparent than

8 Gewin, J. Das Flimmern des Herzens, *Arch. f. Anat. u. Physiol. (Physiol. Section)* **247**, 1906.

9 Hoffa and Ludwig, quoted from Winterberg, H. Studien über Herzflimmern, III, Mitteilung über das Wesen der postundulatorische Pause, *Arch. f. d. ges. Physiol.* **128** 471 (July 10) 1909.

10 Winterberg (Footnote 9).

real Had we been able to determine the exact time of the last stimulation of the pacemaker during each paroxysm and measured from that point to the succeeding P wave, possibly a more constant relationship would have manifested itself

CLINICAL SIGNIFICANCE

In general, paroxysmal forms of auricular fibrillation appear to be an earlier clinical manifestation occurring in less damaged hearts than continuous fibrillation. With few exceptions, the etiology of the two conditions is identical and patients that show recurring paroxysms are to be expected finally to develop continuous fibrillation unless the cause of the arrhythmia is controlled.

Continuous fibrillation does not occur in healthy hearts nor apparently very often in slightly damaged hearts. Paroxysmal or transient fibrillation sometimes occurs in hearts not so seriously damaged and occasionally in apparently healthy hearts that have been subjected to some temporary insult. A case has been reported by Robinson¹¹ in which transient fibrillation followed the inhalation of hydrogen sulphid, although no evidence of heart disease was discovered. I have observed transient fibrillation in two patients with apparently healthy hearts, one a young man admitted to the University Hospital with virulent acute appendicitis and the other, also a young man, who had first indulged freely in alcoholic beverages of questionable purity and then engaged in friendly wrestling bouts.

The lack of data regarding intermittent fibrillation does not permit speaking with any degree of certainty as to its clinical significance. The highly fibrillary state evidenced by the rapidly recurring paroxysms and the fact that our patient quickly developed continuous fibrillation suggests that the condition of the heart muscle is more closely related to that of continuous fibrillation than the ordinary clinical forms of paroxysmal fibrillation.

• SUMMARY

In the case reported, on one examination a condition of fleeting, rapidly recurring paroxysms of auricular fibrillation was found, and on all subsequent examinations, continuous fibrillation. Attention is called to the fact that for the production of this peculiar form of paroxysmal arrhythmia the heart muscle must be not only in a highly fibrillary state, but also at the same time the functional properties of the muscle must be such as to preclude the perpetuation of any of the paroxysms.

¹¹ Robinson, G. C. Transient Auricular Fibrillation in a Healthy Man Following Hydrogen Sulphid Poisoning, *J. A. M. A.* 66:1611 (May 20) 1916.

Auricular extrasystoles were concerned in the onsets of all paroxysms of fibrillation recorded. This is the usual mode of entrance of clinical circus movement, although occasionally it may be initiated by a sinus beat.

Evidence is presented to show that an identical devious pathway was followed by the excitatory process in the various brief paroxysms recorded. This new observation proves that the excitatory process in auricular fibrillation may proceed, for a brief period at least, according to plan and not in a haphazard manner, and is evidence of the fact that there may be but one central or mother wave and not a number of separate fibrillary waves.

The offsets were abrupt and followed by postundulatory pauses of variable duration. It is suggested that the chief factor in determining the position of the succeeding sinus beat is the time of subsidence of the fibrillary excitation in the normal pacemaker, with a relationship similar to that obtaining in the case of auricular extrasystoles.

It is suggested that the form of paroxysmal fibrillation described be called intermittent auricular fibrillation. This condition is apparently closely related to continuous or chronic fibrillation.

ROENTGEN-RAY TREATMENT OF THE SPLEEN IN ASTHMA BRONCHIALE

PRELIMINARY REPORT^{*}

GEORGE L WALDBOTT, M D

DETROIT

LYMPHATIC SYSTEM AND ANAPHYLAXIS

The question whether or not bronchial asthma can be influenced by roentgen-ray treatment of the spleen is suggested by the following considerations. There is a definite relationship of the lymphatic system, of which the spleen is one of the most important organs, to anaphylaxis. Symmers¹ holds the view that anaphylaxis is the cause of sudden death in status thymicolymphaticus. The enlargement of the thymus and the lymphatic tissue that is typical for this disease is regarded by Brown² as the expression of the abnormally sensitive reaction of the body to foreign proteins. We also know that status thymicolymphaticus is frequently associated with anaphylactic diseases, such as urticaria, asthma, eczema, migraine, hay fever and spastic constipation³. On the other hand, in some of the anaphylactic conditions changes in the blood picture are found which resemble very much those occurring in status thymicolymphaticus, namely lymphocytosis and eosinophilia. There is reason to believe that the calcium content of the blood is decreased in anaphylaxis as well as in status thymicolymphaticus,⁴ although definite proof is still lacking.

Some authors⁵ maintain that the good results of thymus irradiation in thymic asthma⁶ are partially due to the stimulation of the lymphatic system exerted by this treatment, and not merely to the mechanical effect of the removal of the thymus tumor. Whether the same reason-

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1 Symmers, D. Status Lymphaticus, *Am J M Sc* **156** 48 (July) 1918

2 Brown, W. L. Principles of Internal Secretion, *Brit M J* **2** 687 (Nov) 1920

3 Henke, F. Der jetzige Stand der Lehre vom Status thymicolymphaticus und seine Beziehungen zu anderen Krankheiten, *Deutsch med Wchnschr* **46** 1257 (Nov) 1920

4 Grove, W. R., and Vines, H. W. C. Calcium Deficiencies, Their Treatment by Parathyroid, *Brit M J* **1** 791-795 (May) 1922. Pottenger, F. M. The Physiologic Basis for the Employment of Calcium in the Treatment of Asthma Paroxysms, *Calif State J Med* **21** 293-294 (July) 1923. Novak, F. J., and Hollander, A. R. A New Treatment for Hay-Fever Hyperesthetic Rhinitis, and Bronchial Asthma, *Illinois M J* **45** 285 (April) 1924

5 Chelmonski, A. Zur Pathogenese des Asthma Bronchiale, *Deutsch Arch f klin Med* **105** 522, 1912. Klewitz, D. F. Roentgenbestrahlung bei Asthma Bronchiale, *Munchen med Wchnschr* **69** 305-306 (March) 1922

6 Morgan, H. J., and Dachtler, H. W. Thymic Asthma Successfully Treated by X-Rays, *Surg, Gynec & Obst* **19** 784, 1914

ing may be applied to the explanation of the great benefit from roentgen-ray treatment of the hilum glands in asthma is a question that has not yet received the necessary attention in literature

SPLEEN AND ANAPHYLAXIS

Experimental Evidence—In regard to the part that the spleen takes in anaphylaxis, the work so far reported indicates that the spleen participates in the production of antibodies. This is demonstrated by the following facts. Antibodies are detected in the spleen earlier than in the blood.⁷ Antigens have been found localized in the spleen.⁸ Cultures of splenic tissue outside the body may produce antibodies in the presence of antigenic substances.⁹ The concentration of antibodies in the spleen is greater than in the blood.¹⁰

In recent years Hektoen¹¹ showed that removal of the spleen reduces the power of an animal to produce antibodies, whereas irradiation of the spleen seems to increase the antibody titer of the blood. Motohashi¹² confirms the results of Hektoen in regard to splenectomy.

Clinical Evidence—This idea of Hektoen of stimulating the antibody production by means of roentgen-ray treatment of the spleen was used as the basis for some clinical studies on a small number of asthma patients during the last winter. Most of these patients are definitely improved. Owing to the short time that has elapsed since the beginning of the treatment, the details will be given in a later paper. One case, however, shows such a marked improvement that it justifies a presentation of a detailed account.

Moreover, further support is given to this means of treatment by some German authors,¹³ who reported the following results on seventy-one treated cases of bronchial asthma: questionable, ten; negative, seven; good, fifteen; very good, twelve; and splendid, twenty-seven.

7 Tsurumi, M., and Kohda, K. The Origin of the Complement Fixing Antibodies, *Ztschr f Immunitätsforsch u exper Therap* **19** 519-528, 1913.

8 Cary, W. E. The Fate of Foreign Erythrocytes Introduced into the Blood Stream of the Rabbit, *J Infect Dis* **17** 432, 1915. Luckhardt, A. B., and Becht, F. C. The Role of the Spleen in the Fixation of Antigen and the Formation of Antibodies, *Tr Chicago Path Soc* **8** 202, 1911.

9 Carrel, A., and Ingebrigtsen, M. Antibody Production by Living Tissue Outside the Organism, *Compt rend Soc de biol* **72** 220, 1912.

10 Jatta. *Ztschr f Hyg u Infektionskrankh* **33** 185, 1900.

11 Hektoen, L. Further Observations on the Effects of Roentgenization and Splenectomy upon the Production of Antibodies, *J Infect Dis* **27** 23-30 (July) 1920.

12 Motohashi, S. The Effect of Splenectomy upon the Production of Antibodies, *J M Res* **43** 473 (Aug-Oct) 1922.

13 Groedel, F. M., and Lossen, H. Die Röntgenbehandlung des Asthma bronchiale, reprint of chapter from Salzmann, F. Die Roentgenbehandlung innerer Krankheiten, Munich, J. F. Lehmann, 1922.

Pohlman,¹⁴ following Groedel's work, states that he treated forty-five cases of asthma, among which only four did not respond to treatment. All others showed distinct improvement. None of the cases of Pohlman or of Groedel has been studied clinically.

REPORT OF CASE

History—H D, a white American boy, aged 6½ years, was admitted to the outpatient department of the Children's Hospital, Sept 22, 1924, on account of asthma. Two brothers and one sister were in perfect health, and no anaphylactic disease could be traced in the family. The child's history recorded pneumonia about two years before admission, and among the usual children's diseases, chickenpox and measles. The boy had severe urticaria three times, the last time one year before. Nearly every fall he had considerable discharge from the nose and was troubled with sneezing.

The first asthma attack dated back four years, the attacks had become more severe and more frequent, recently occurring as often as once in one or two weeks. In the evenings as a usual thing the child suddenly became dyspneic and cyanotic, and many wheezing noises were heard. The attack usually lasted from one to two hours. No vomiting occurred. It could not be found from the history whether food allergy or exposure to any anaphylactogen substance produced the seizures. The boy did not observe any special regulations in his diet, he disliked milk. There was no dog or cat in his home. He did not sleep in a feather bed. In regard to bacterial anaphylaxis, there was no tonsillitis and no sinusitis. A few cavities of the teeth had been noticed during recent months.

Examination—Physical examination made in an interval between attacks revealed a well nourished, very active boy, who was slightly orthopneic. The skin and mucous membranes showed a good color, no cyanosis being present. A slight dermatographism was noticeable. A few not sensitive submaxillary glands were felt. The eyes, the ears and the sinuses were negative. The nose showed a considerable watery discharge. The tongue was not coated, the teeth were somewhat ground down and showed a few cavities. The tonsils were not enlarged but were somewhat adherent, no pus could be expressed. The chest suggested a slight barrel shape, the excursions were somewhat embarrassed. The heart dulness was small 3.5 cm to the left in the fifth interspace and 0.5 cm to the right in the third interspace. The sounds were clear though slightly distant, the pulse was regular, full and of normal pressure (112 systolic, 83 diastolic). The lungs showed hyperresonance throughout, the bases descended fairly well, expiration was prolonged, and there were a few bronchitic bruits, especially around the hilum region, but no wheezing sounds. The abdomen, spleen, liver, extremities, genitalia and nervous system did not reveal anything unusual.

Laboratory Findings—The urine was negative for albumin and sugar and on microscopic examination the red blood cells totaled 4,390,000, the white blood cells, 7,800, hemoglobin, 87 per cent, polymorphonuclears, 45 per cent, small lymphocytes, 47 per cent, large lymphocytes, 4 per cent, and eosinophils, 4 per cent. The Wassermann reaction was negative. The blood calcium was 8.5 mg. The sputum expectorated during an attack and examined in my office showed many eosinophil cells and Charcot-Leyden crystals.

Among the skin tests done we found milk ++, casein +, eggs, whole, ++, and wheat +. Other tests were negative.

Roentgen-ray examination disclosed a congenital deformity of the third right rib on the sternal portion. The lungs showed extensive bilateral root infiltration with a tendency to extend along the bronchi.

¹⁴ Pohlman, C. Milzebestrahlung bei Bronchial Asthma, München med Wchnschr 72 57-58 (Jan) 1925.

Course of Disease—September 25, the child received one roentgen-ray treatment of the spleen from Dr Evans, with the following technic 6 minutes, 5 milliamperes, 4 mm A1, 88 kilowatts, 10 inch skin target distance. He was told to observe antianaphylactic diet for two weeks and then no longer to restrict his diet. October 2, he felt very much prostrated, vomited a few times and was slightly dyspneic. October 25, examination of the chest was completely negative. On physical examination, however, no change in the roentgen-ray findings was seen. During the winter months the child had slight bronchitis twice, lasting from two to five days, this cleared up spontaneously. There was not a single attack of asthma after the treatment.

Examination of the chest, May 20, 1925, showed no cyanosis and no orthopnea. Expiration was not prolonged, there was no tympany, and no râles or bruits were heard. The heart dulness was normal. The red blood cells totaled 5,200,000, the white blood cells, 9,400, hemoglobin, 100 per cent, polymorphonuclears, 71, small lymphocytes, 29, large lymphocytes, 8, and eosinophils, 2. The blood calcium was 15.4. The formerly positive skin tests were negative. The sputum examination was negative for tuberculosis, eosinophils, Curschmann spirals and Leyden crystals. There was no change in the roentgen-ray findings.

COMMENT

The diagnosis asthma bronchiale on an anaphylactic basis does not need any further comment in view of the typical history, of the physical, laboratory and roentgen-ray findings and of the skin tests. The striking feature of this case is the complete cessation of the apparently very severe and frequent asthma attacks during eight months' observation. It is to be noted that the blood calcium rose slightly, the eosinophil cells decreased and the skin tests became negative, while the root infiltration in the roentgenogram did not change on repeated examinations after the treatment. It cannot yet be stated whether the effect of this treatment was due to the psychic influence, as frequently happens in this disease, or whether it conformed with the previously mentioned conception that a stimulation dose of roentgen ray of the spleen may increase the antibody content of the organism. However, similar experiences with other cases seem to indicate that the skin tests become negative within about one week after the treatment. Perhaps the prostration and nausea, which came on seven days later in the foregoing case, may be the main curative factor. Further experimental and clinical studies on this subject are being carried out at present.

SUMMARY

One patient with asthma bronchiale treated with a small dose of roentgen ray over the spleen has been entirely relieved from the clinical manifestations of this disease. The quoted literature, as well as the favorable results obtained on subsequent cases, call for further investigation in this matter.

THE NONSPECIFIC PROTEIN REACTION¹

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A recent series of articles¹ in the ARCHIVES OF INTERNAL MEDICINE merits considerable comment and criticism. Ling attempts to "study comparatively the influence of various nonspecific protein agents on the reactive temperature, on the mobilization of peripheral blood leukocytes, antibodies and enzymes." With the crude mixtures—horse serum, milk, peptone, vaccines, yeast, crotalin—used by him it is obvious that the results obtained cannot be arbitrarily saddled on any one constituent, and also that any attempt to evaluate the results quantitatively is useless.

Ling admits in his article that distilled water produces similar reactions to those produced by the protein mixtures. It was shown in a series of articles² in the *American Journal of Physiology* that distilled water, even if it has been sterilized, is capable of producing considerable fever when injected intravenously, and this is because the water had at some time been contaminated by bacteria carried to it by dust in the air or in some other way. These bacteria are able to survive and in fact reproduce in this presumably pure water and may be found in a viable state two months and more after the initial inoculation. They themselves may be killed during sterilization but a soluble product which they have produced during their lifetime and which filters through the Berkefeld candle is heat stable and remains in the sterile water. It is this product, so far unidentified but containing nitrogen, which is responsible for the fevers produced when the waters are injected. The only method so far outlined for removing the fever producing material from water is by distillation. If, however, the distillate is sterilized immediately, it can be kept indefinitely in its pure state. The type and intensity of the fever may be varied in a quantitative manner, according to how much of the bacterial products and what bacterial products are injected.

¹ From the Department of Pathology and the Otho S. A. Sprague Memorial Institute, University of Chicago.

1 Ling, C. Y. Mechanism of Reaction of Nonspecific Protein Agents in Treatment of Disease, I, Influence of Various Agents on Temperature and Leukocyte Counts in Normal Persons and in Rabbits, *Arch Int Med* **35** 598-608 (May) 1925, II, Influence of Various Agents in Mobilization of Blood Antibodies, *ibid* **35** 740-751 (June) 1925.

2 Seibert, F. B., and Mendel, L. B. Temperature Variations in Rabbits, *Am J Physiol* **67** 83-89 (Dec.) 1923. Seibert, F. B. Fever Producing Substance Found in Some Distilled Waters, *ibid* **67** 90-104 (Dec.) 1923, Protein Fevers, *ibid* **67** 105-23 (Dec.) 1923, Cause of Many Febrile Reactions Following Intravenous Injections, *ibid* **71** 621-651 (Feb.) 1925.

Moreover, specific antibodies were produced by immunizing rabbits with one of the contaminated distilled waters to such an extent that serum diluted 1 8,000 agglutinated a strain of bacteria isolated from the water

In connection with the study of protein fevers, it was shown that casein when prepared in such a manner as to eliminate bacterial contamination did not produce fever when injected into rabbits, whereas all caseins prepared in the usual manner did produce fever. Whether or not it is the protein of the bacteria contaminating the distilled waters that is responsible for the temperature reactions is still an open question, but the presence of nitrogen in the fever producing waters has been demonstrated

Therefore, in view of these clearly established facts, one cannot evaluate the nonspecific effect of such mixtures as horse serum, milk, peptone, vaccines, yeast, etc., and expect similar results with different preparations of the same substance. The results obtained may possibly be due to contaminating substances and not at all to the agent specified

Ling's statement that "*Bacillus typhosus*, peptone and market milk gave the most prompt initial temperature reaction, while that produced by market milk seemed to persist longer than that of other agents" may possibly find an interpretation in the fact that different bacteria among the large group that contaminates water vary in just this manner in the reactions produced by them

Book Reviews

PATHOGENIE DES CALCULS BILIAIRES ET INDICATIONS OPERATOIRES By THORKILD ROVSING Price, 20 francs Paris Masson & Cie

This work originally appeared in Danish in 1922, and this French translation is made for the purpose of giving the theories of the author wider publicity. It is frankly a piece of special pleading and as such is ably done, but can hardly be said to be a fair or systematic review of our knowledge of the pathogenesis of gallstones.

The first part of the book is taken up with a critique of the studies of Naunyn and his collaborators on the rôle of infection in the production of calculi. Rovsing is unable to accept any theory that sepsis or biliary stasis are etiologic factors, mainly for the reason that no adequate experimental basis can be offered for this theory. Enterogenous infection is mainly discussed and no mention is made of the work of Rosenow in the production of calculi by the intravenous injection of bacteria.

Aschoff and his school have done a tremendous work on the rôle of cholesterol in stone production. This is reviewed and also found wanting. The word diathesis is used frequently, but the recent discoveries of the chemists in relation to lipid metabolism are ignored.

Rovsing's theory is that in many toxemias there is a deposition in the liver of minute particles of a calcium compound of bilihumin. These particles are exceedingly irregular and branched and hence readily clump. They can be demonstrated microscopically in the liver and in the bile in pregnancy and in acute toxemias of diverse origin. Most of these pass out into the bowel but some find their way into the gallbladder, where they accumulate in the fundus as they are heavy. As the bile is concentrated in the gallbladder by the absorption of water they are surrounded with deposits of other biliary elements, chiefly cholesterol. There is a network of protein derived from mucus or blood in most stones. Therefore, infection, stasis or cholesterol are not factors at all. This theory is elaborately worked out and much evidence in its support produced. However, it cannot be said to be any more definitely proved than the ones discussed earlier in the book.

It is singular that the book treats mostly of stones as isolated phenomena, and not of gallbladder disease as a whole. Perhaps more might be gained from the study of gallbladders than of the stones they contain. McCarthy's work on this subject is not mentioned.

MEDICAMENTS ET MEDICATIONS CARDIAQUES PAR H. VAQUEZ Leçons recueillies par M. Theodoresco Pp 302 Paris J. B. Baillière et fils, 1925

This volume consists of a series of lectures on the treatment of heart disease by Vaquez, designed for his students and edited by Theodoresco. The first half of the volume is given over to a discussion of cardiac remedies, especially digitalis and its allies. It is pleasing to find that the author has seen fit to give as much of the historical background as he has. This is especially true in the case of digitalis. An understanding of what was accomplished by the clinical observations of the earlier investigators should make us all properly humble. This is especially true of Withering, who worked without the recorded experience of others, having only his own observation and deduction to guide him.

After the chapter on the history of digitalis, the drug is taken up in detail, its physiologic action, indications for its use, method of using and dosage. The preparations of strophanthus, especially ouabain, receive more attention.

than in American texts. Quinidin is given a full chapter. Under the heading of "Minor Cardiac Remedies" a large number of drugs little used here are considered. The pharmacology does not always coincide with the generally held American and English views, and there is more of therapeutic optimism that is usual in English and American works. The differences in point of view, however, cannot be other than stimulating.

The second half of the volume is taken up with the treatment of cardiac conditions, beginning with a chapter on the hygiene of the cardiac patient, under which are included exercise, diet, and such questions as marriage, military service and anesthesia. Then follow chapters on the treatment of the various cardiac disorders.

It is a decided advantage, in view of the wideness of the field of cardiac knowledge, to have a volume devoted to treatment only. The manner of approach, the clear diction, and the availability afforded by the systematic arrangement add to the value. The author is too well known to make necessary any comment on the scientific worth of the work. It is unfortunate that a reading knowledge of French is not general enough to make books of this kind more generally known and used, or that unedited translations are not more frequent.

LES VAISSEAUX LYMPHATIQUES DU COEUR CHEZ L'HOMME ET CHEZ QUELQUES MAMMIFERES. By OTTO C. AAGAARD, Ancien Chef de Clinique Chirurgicale a la Faculte de Copenhague. Pp 478, 152 illustrations. Copenhagen Levin & Munksgaard.

This well written and beautifully illustrated monograph is based partly on the literature and partly on the author's own work. About 200 human hearts were used in this study and comparative observations were made on hearts of other mammals. The lymphatics were rendered visible by the injection method. Many illustrations of historical interest are reproduced.

The monograph is purely anatomic and was presented to the faculty of medicine as a thesis for the degree of doctor of medicine. If this monograph is the type of scholarly requirements for the degree of doctor of medicine in Denmark, that little country has apparently something to teach us in this direction. It is to be regretted that the monograph is so exclusively anatomic. It would have been more valuable if the problems on the significance of the heart lymphatic system in the various conditions of heart disorders also had been considered. It is apparently well established from this work of Aagaard and from that of others that all regions of the heart, including the endocardium and the epicardium, are richly supplied with lymphatics. In man the lymphatic supply is richer in the myocardium of the ventricles than in the myocardium of the auricles.

The publication of the monograph in French renders it less valuable to English speaking physiologists and internists interested in cardiovascular physiology and pathology than it would be if written in English. There is a short resumé in Danish, but no index.

THE ANTIGENIC PROPERTY OF POLLENS *

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Since Wolff-Eisner¹ suggested in 1906 that the symptoms of pollen disease resemble the phenomena of experimental anaphylaxis, the relationship of these conditions has been extensively studied clinically and experimentally. Soon after 1906 Billard and Maltet,² Meltzer,³ and Billard⁴ suggested that there were certain clinical and laboratory similarities of these conditions, while many others submitted experimental evidence to support this view. Dunbar,⁵ Koessler,⁶ Heyl,⁷ Alexander,⁸ Harrison and Armstrong⁹ and Parker¹⁰ reported the demonstration of severe or fatal anaphylactic symptoms following the injection of pollen extracts into specifically sensitized guinea-pigs. Parker,¹¹ using the uterine strip method, demonstrated that active sensitization of guinea-pigs with pollen extracts can be accomplished, an observation that was to be anticipated considering the protein content of pollens. Dunbar, Koessler and Alexander reported successful passive sensitization of normal guinea-pigs by injecting into them the blood serum of patients suffering from pollen disease or of guinea-pigs sensitized with pollen extracts. There also are numerous clinical reports

¹ From the Otho S. A. Sprague Memorial Institute and the Department of Pathology, University of Chicago.

^{*} A preliminary report of this paper was given at the meeting of the American Association for the Study of Allergy, Chicago, 1924.

1 Wolff-Eisner. *Das Heufieber*, Munchen, 1906.

2 Billard G, and Maltet L. *J de physiol et de path gen*, 1907, p. 304.

3 Meltzer, S. J. *J A M A* **55** 1021 (Sept 17) 1910.

4 Billard, G. *Lancet*, 1910, pp. 179, 1208.

5 Dunbar, W. P. *Deutsch med Wchnschr* **37** 578, 1911.

6 Koessler, K. K. *Forchheimer's Therapeutics of Internal Diseases* **5** 6, 1914.

7 Heyl, F. W. *Tr Am Chem Soc* **39** 1470, 1917.

8 Alexander, M. E. *J Immunol* **8** 457 (Nov) 1923.

9 Harrison, W. T., and Armstrong, C. *Pub Health Rep* **39** 1261 (May 30) 1924.

10 Parker, Julia T. *Proc Soc Exper Biol & Med* **18** 237, 1920-1921, *J Immunol* **9** 515 (Nov) 1924.

11 Parker (Footnote 10, first reference).

of more or less severe anaphylactic-like symptoms in man following the prophylactic use of small doses of pollen preparations

On the other hand, Cooke, Flood and Coca¹² in well controlled experiments were not able to demonstrate definite anaphylactic phenomena, passive sensitization or the production of specific antibodies. Because of this failure to show the antigenic property of pollen extracts, Cooke and his associates and Coca have attempted to rule pollen disease out of the category of anaphylactic phenomena. Coca¹³ divided all cases of hypersensitiveness into two groups, the anaphylactic and the allergic, and places pollen disease under the allergic group.

Zinsser¹⁴ recently stated his view of the question as follows: "While therefore we must again call attention to the important difference between true anaphylaxis and hay-fever because of the probable rôle of inheritance, we can also emphasize the many points of definite analogy between the two conditions."

In earlier studies of pollen disease we had observed that the sensitization of guinea-pigs with pollen extracts was not easily accomplished. Because of this observation and because of the conflicting reports in the literature we planned a series of experiments to study the antigenic properties of pollen extracts. Our methods are given as developed.

PREPARATION OF POLLEN EXTRACTS

The pollen extracts were all prepared in the same manner except for variations in sodium chlorid content and in ratio of pollen to extracting solution. The dried pollens were weighed and placed in sterile thick walled bottles, which contained small irregular glass beads. These bottles were then shaken in a shaking machine until the pollen grains were fairly completely macerated, as determined microscopically. The macerated pollen was then extracted at room temperature in the shaking machine with a chloroform saturated 0.85 per cent sodium chlorid solution¹⁵ that contained 0.25 per cent sodium bicarbonate solution. The ratio of dry pollen to extracting solution varied from 1:10 to 1:30 depending on experimental needs.

After from twenty-four to thirty hours' extraction, the solutions were allowed to settle, centrifugated at high speed, decanted, filtered through Berkefeld filters, and after sterility had been proved anaerobically and aerobically were sealed in glass and kept in an icebox until used. The nitrogen content of all extracts was determined by the Kjeldahl method.

¹² Cooke, R. A., Flood, E. P., and Coca, A. F. *J. Immunol.* **2**: 217 (Feb.) 1917.

¹³ Coca, A. F. *Text-Book of Practice of Medicine* **1**: 179, 1920.

¹⁴ Zinsser, H. *Infection and Resistance*, Ed. 3, pp. 487-489.

¹⁵ Extract A used in the first experiment was extracted with 8.5 per cent sodium chlorid solution.

ANAPHYLACTIC STUDIES

The usual method used in demonstrating experimental anaphylaxis was employed. Two extracts of the pollen of *Ambrosia trifida* (giant ragweed) were prepared, *A* containing 8.5 per cent sodium chloride solution, and *B* 0.85 per cent sodium chloride solution, both with the dilution of 1 gm of pollen to 10 cc of extracting solution. The nitrogen content of *A* was 1.61 mg per cubic centimeter and of *B* 1.54 mg per cubic centimeter.

Six guinea-pigs weighing from 150 to 500 gm were injected intraperitoneally with 1 cc of solution *A* (1.61 mg of nitrogen) diluted to 10 cc with water. After an interval of from eighteen to twenty-two days, from 5 to 10 cc of solution *B* (7.7–15.4 mg of nitrogen) warmed to body heat was injected intraperitoneally. All the pigs showed varying degrees of such signs as restlessness, scratching of the nose

TABLE 1—*Nonfatal Anaphylaxis in Guinea-Pigs with Pollen Extracts**

Animal	Wt, Gm	Date of Injection	Amount Injected	Date of Shocking Injection	Amount Injected	Results
3	450	2/ 8/23	1 cc = 1.61 mg N	2/27/23	5 cc = 7.7 mg N	Slight symptoms
9	420	2/13/23	1 cc = 0.80 mg N	3/ 5/23	10 cc = 15.4 mg N	Slight symptoms
13	460	2/ 7/23	1 cc = 0.80 mg N	2/26/23	10 cc = 15.4 mg N	Slight symptoms
17	500	2/ 7/23	0.5 cc = 0.80 mg N	2/27/23	5 cc = 7.7 mg N	Slight symptoms

* Sensitizing and shocking doses given intraperitoneally. Similar results were obtained with other pigs.

TABLE 2—*Nonfatal Anaphylaxis in Guinea-Pigs with Pollen Extracts**

Animal	Wt, Gm	Date of Injection	Amount Injected	Date of Shocking Injection	Amount Injected	Results
5	430	2/21/23	1 cc = 1.61 mg N	3/13/23	1 cc = 1.54 mg N	Slight symptoms
11	450	2/21/23	1 cc = 1.61 mg N	3/13/23	1 cc = 1.54 mg N	Slight symptoms
19	300	3/ 7/23	1 cc = 1.61 mg N	3/26/23	1 cc = 1.54 mg N	Slight symptoms

* Sensitizing dose given intraperitoneally, shocking dose given intracardially. Similar results were obtained with other pigs.

and ears, elevation of the hair on the neck and back, urination, defecation, etc., but no death occurred. The normal pigs gave no evidence of a reaction (Table 1).

Six additional pigs were given a sensitizing dose similar to that used before, and after an interval of from eighteen to twenty-two days were injected intracardially with 1 cc of extract *B*. The results were similar to those obtained in the first group. No deaths occurred. The normal pigs gave no evidence of a reaction (Table 2).

After these failures to produce fatal experimental anaphylaxis with pollen extracts in guinea-pigs by the usual methods, we gave six young virgin guinea-pigs, weighing about 150 gm, two or three intraperitoneal

findings characteristic of anaphylaxis, four died before two hours with a gradually developing paralysis, and one, although very sick for a time, survived. Of six pigs that had received three sensitizing doses, three died within eight minutes after receiving the injection and three died within two hours. Of the twelve pigs, five died within eight minutes, six more died within two hours, and one pig survived. Four normal pigs of the same lot gave no symptoms following an intravenous injection of from 1 to 2 c c of the same pollen extract. We next sensitized six pigs weighing about 250 gm by the same method and obtained in three pigs, after an intravenous injection of 1 c c of pollen extract, typical bronchoconstrictions as demonstrated by the graphic method reported in 1924 by Koessler and Lewis at the Buffalo meeting of the Association of Pathologists (Figs 2 and 3). Normal pigs gave no reaction by this method.

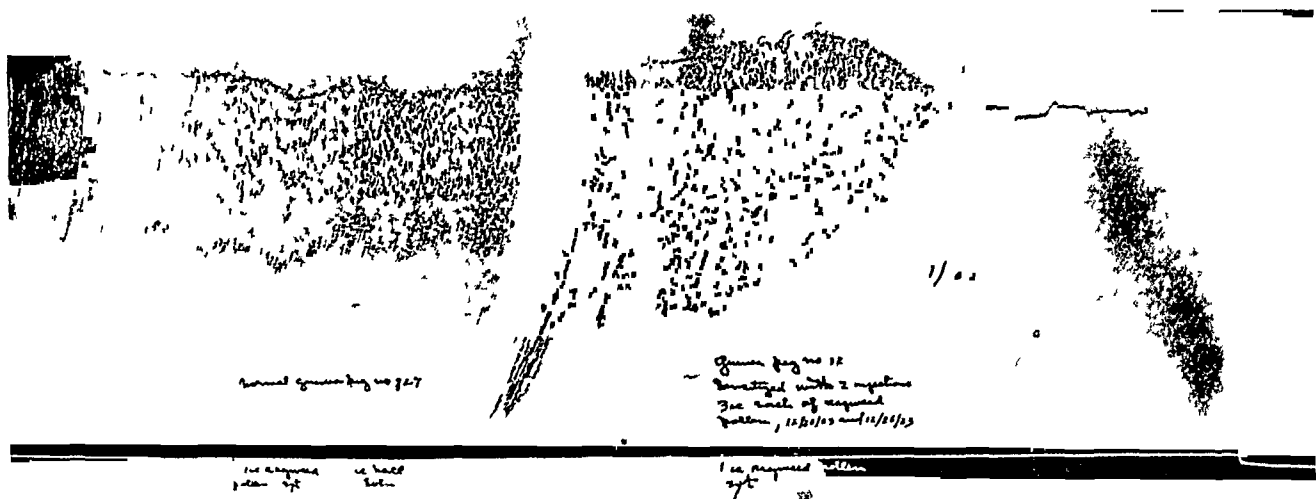


Fig 3—Record of bronchoconstriction and typical anaphylaxis after injection of *Ambrosia trifida* pollen

As several reports of successful passive sensitization to pollens have appeared, we sensitized nine pigs by the method described above for this purpose. Nine days following the last injection, the pigs were bled and the blood serum was transferred to an equal number of normal pigs. These pigs were tested from twenty-four to forty-eight hours later by three methods. (1) Three pigs were injected intravenously with 1 c c of pollen extract and all were uncomfortable and restless for an hour or more and then appeared normal, (2) three virgin pigs were killed and their uteri gave typical, although moderate, contractions when tested with the pollen extract (Fig 4), and (3) two of three pigs gave moderate bronchial contractions when injected intravenously with 1 c c of pollen extract.

ANTIBODY PRODUCTION

Four rabbits were given at three day intervals from twelve to twenty-five intravenous injections of *Ambrosia trifida* (giant ragweed) pollen extract prepared as described above. The first injection contained 0.5 mg of nitrogen, and each succeeding injection was increased so that the twelfth contained 3.5 mg of nitrogen. This dose was continued until the termination of the experiment. From five to six days after the last injection, the three surviving rabbits were bled and the serum tested for precipitins and complement fixing antibodies. The serum from all immunized rabbits contained precipitins for the pollen extract (Table 4) and showed complete inhibition of hemolysis by the complement fixation test. Normal rabbit serum gave negative results for both tests.

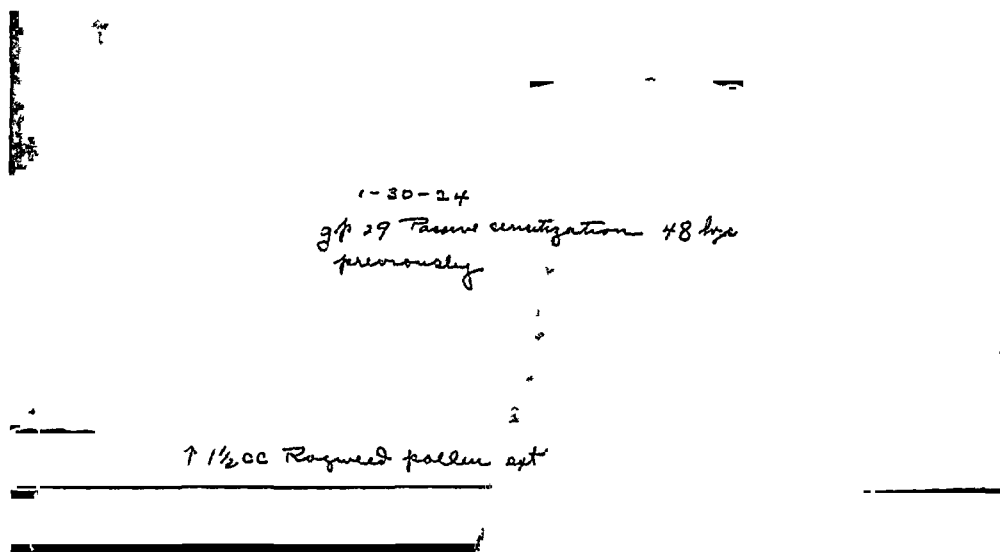


Fig 4—Record of passive anaphylaxis to *Ambrosia trifida* pollen extract, guinea-pig to guinea-pig (uterine strip method)

SPECIFICITY OF POLLENS

In 1873 Blackley¹⁶ published the results of his extensive experiments to prove that pollens were the cause of the symptoms called "hay-fever" and that experimental contact showed a certain degree of specificity. He says

And I have also shown that, with very rare exceptions, this power is common, in some degree, to the pollen of almost all the plants experimented with. And although those belonging to the order *Graminaceae* have this property in a more marked degree than some, there are plants belonging to other orders which have it to almost, if not quite an equal extent.

¹⁶ Blackley, C. H. Experimental Researches on the Cause and Nature of Hay-Fever, 1873.

Since Blackley's time the question of specificity of pollen has been studied extensively, not only by observing the effect of experimental contact, but also by other methods, such as ophthalmic reactions, skin sensitization and therapeutic tests. It is supposed that, if pollens from one member of a so-called group give a positive reaction in a sensitive person, all pollens from that group should also give positive reactions *regardless of whether the subject has ever had a previous contact with all of them*. Then, too, in discussions on the specific treatment of pollen disease it is commonly stated that extracts from any one pollen of a

TABLE 3—*Fatal Anaphylaxis in Guinea-Pigs with Pollen Extracts* *

Animal	Wt, Gm	Date of Injection	Amount Injected	Date of Shocking Injection	Amount Injected	Results
1	140	12/20/23	3 cc = 3.96 mg N	1/ 7/24	1 cc = 1.32 mg N	Death in 26 minutes
		12/26/23	3 cc = 3.96 mg N			
3	150	12/20/23	3 cc = 3.96 mg N	1/10/24	1.5 cc = 1.98 mg N	Death in 20 minutes
		12/26/23	3 cc = 3.96 mg N			
34	310	1/10/24	2 cc = 2.64 mg N	2/ 5/24	1.5 cc = 1.98 mg N	Death in 2 minutes
		1/14/24	3 cc = 3.96 mg N			
		1/17/24	2 cc = 2.64 mg N			
35	120	1/14/24	2 cc = 2.64 mg N	2/ 7/24	1.5 cc = 1.98 mg N	Death in 2 minutes
		1/17/24	2 cc = 2.64 mg N			
40	180	1/14/24	3 cc = 2.96 mg N	2/ 8/24	2 cc = 2.64 mg N	Death in 20 minutes
		1/17/24	2 cc = 2.64 mg N			
41	240			3/ 7/24	1.5 cc = 1.98 mg N	No symptoms
42	200			3/ 7/24	1.5 cc = 1.98 mg N	No symptoms

* Sensitizing doses given intraperitoneally, shocking doses intravenously. Similar results were obtained with other pigs.

TABLE 4—*Production of Precipitins with Pollen Extracts in Rabbits* *

Rabbit	Date	Number of Injections			Dilution of Pollen Extract				
		Subcutaneous	Intraperitoneal	Intravenous	30	60	120	240	480
2	5/6/24	0	0	0	—	—	—	—	—
2	8/7/24	0	0	21	++	++	+	—	—
3	5/6/24	0	0	0	—	—	—	—	—
3	8/7/24	0	0	16	+++	+++	++	+	—
4	5/6/24	0	0	0	—	—	—	—	—
4	8/7/24	2	2	12	++	++	+	—	—
5	8/7/24	0	0	0	—	—	—	—	—

* Tested before and after injections.

group are as efficacious as those from another member of the same group. The generally accepted explanation for this view is that the chief proteins of the pollens of a group are identical and that symptoms are produced by contact with that protein. In support of this view, Wells and Osborne¹⁷ have definitely shown that, when tested anaphylactically, the chemical relationship of similar tissues of a group parallels the biologic relationship. However, a critical study of many of the

17 Wells, H. G., and Osborne, T. B. J. Infect. Dis. **12** 341, 1913, J. Infect. Dis. **17** 259, 1915.

reports on pollen disease brings to light certain facts that cannot be disregarded. For instance, it has been observed that a certain percentage of all patients with pollen disease, tested in a routine manner with several pollens of a group, while reacting strongly to one or more of the pollens, react poorly or not at all to other pollens of the same group, and again that prophylactic treatment with one pollen extract does not yield the same therapeutic results as that with an extract of another member of the same group.

In recent reports, especially by Selfridge,¹⁸ Watson and Kibler¹⁹ and Bernton,²⁰ exception is taken to the unqualified acceptance of the view of group specificity. They agree that a certain amount of specificity exists, but contend that this conception does not explain satisfactorily many observed irregularities. Parker²¹ has recently presented some experimental evidence to show that a definite specificity exists for the pollens of *Ambrosia elatior* and *Ambrosia trifida*.

With this question before us we began some studies over a year ago to learn more about the specificity of pollens. We found it difficult to evaluate much of the work already reported, as each investigator used his own methods in preparing materials and in making clinical and experimental comparisons. In our work we have used extracts of four of the common pollens, namely, *Ambrosia trifida* (giant ragweed), *Ambrosia elatior* (small ragweed), *Helianthus annuus* (sunflower) and *Zea mays* (corn) collected by one of us (H. L. H.) in the fall of 1922. An extract of each pollen was made by the method described above, and after the sterility was proved anaerobically and aerobically the nitrogen content was determined by the Kjeldahl method. With each pollen extract, six virgin guinea-pigs, about four weeks old and weighing not more than 150 gm., were given one intraperitoneal injection containing about 1.5 mg. of nitrogen. Twenty or more days later, the pigs were killed by a blow over the head, the uterus was removed at once and immersed until used in a modified Locke's solution which was kept at 37° C. and through which a constant stream of washed oxygen was bubbled. The uterine strips were then tested by the Dale method with the nonspecific and specific extracts. Dilutions up to 1:5,000 with a nitrogen content of about 0.002 mg. per cubic centimeter, except as noted below, gave typical contractions as soon as the specific extract was added, while with the exception of the extract of *Zea mays* pollen much lower dilutions gave no reaction with the normal uterine strip.

18 Selfridge, G. Calif. State J. Med. **17** 106 (April) 1919, *ibid* **17** 139 (May) 1919.

19 Watson, S. H., and Kibler, C. S. Etiology of Hay-Fever in Arizona and Southwest, J. A. M. A. **78** 719 (March 11) 1922.

20 Bernton, H. Treatment of Seasonal Hay-Fever, and Possible Causes of Failure, J. A. M. A. **80** 1301 (May 5) 1923.

21 Parker (Footnote 10, second reference).

When tested with the nonspecific extract the sensitized uterus gave little or no response, except to the extract of *Zea mays* pollen. The pollen from the two ambrosias gave small nonspecific reactions when tested against each other and marked reactions when tested specifically (Figs 5 and 6). This difference has been noted many times clinically while making the routine skin sensitization tests with these two pollens. All uterine strips, normal, nonspecifically sensitized and specifically sensi-

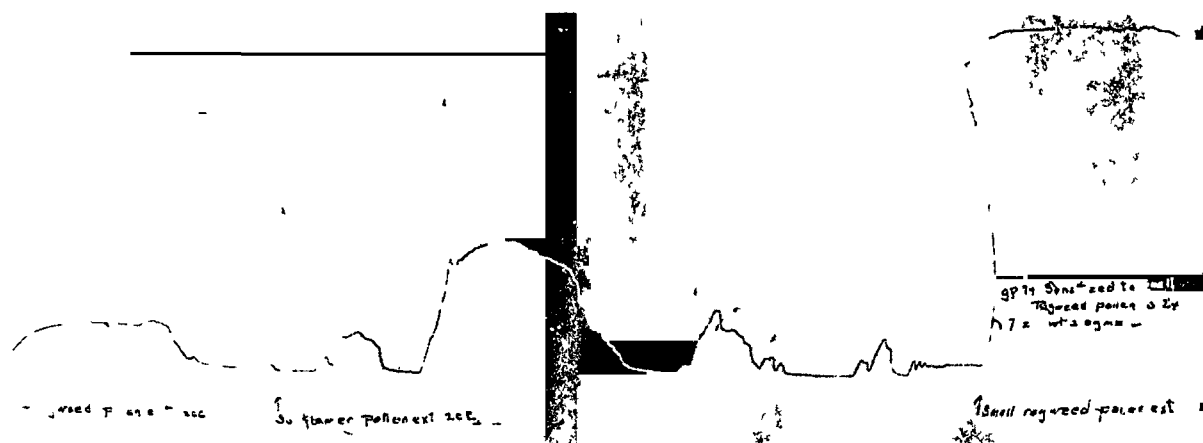


Fig 6—Record of active anaphylaxis to *Helianthus annuus* (sunflower) pollen extract in guinea-pig (uterine strip method)

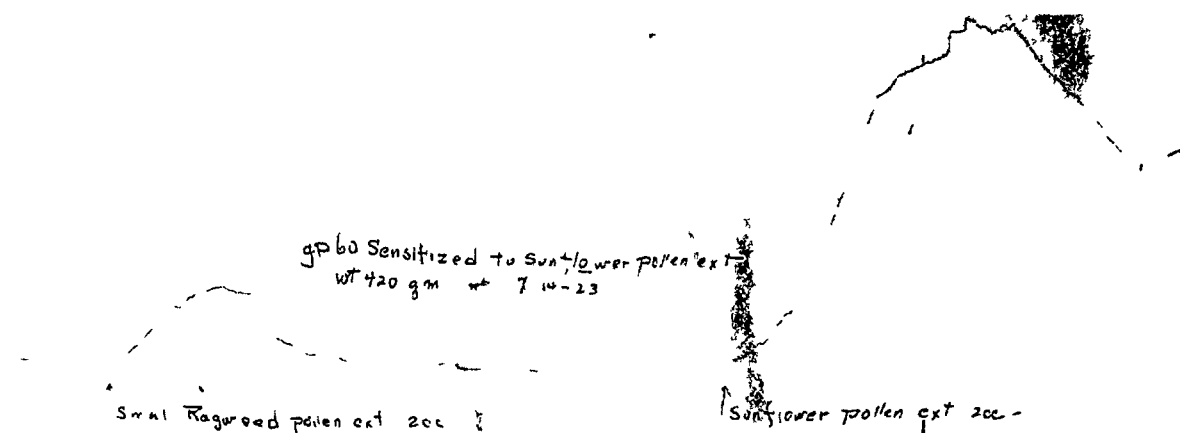


Fig 6—Record of active anaphylaxis to *Helianthus annuus* (sunflower) pollen extract in guinea-pig (uterine strip method)

tized reacted vigorously to the extract of *Zea mays* pollen in dilutions as high as 1:30,000 or about 0.0002 mg of nitrogen per cubic centimeter (Fig 7). Clinically we have noted also that many patients at least give small positive skin reactions to the pollen of *Zea mays* regardless of whether they give reactions to any other pollen. Corn pollen contains therefore an oxytocic principle.

COMMENT

This experimental evidence, showing that by using pollen extracts as an antigen characteristic anaphylactic reactions can be elicited in the guinea-pig and that antibodies can be demonstrated in the blood of properly sensitized experimental animals, strengthens the view that pollen disease may be placed in the group of anaphylactic disorders, and suggests that this phase of the problem should be considered by the clinician when he institutes prophylactic measures for the relief of symptoms. Our experience, as well as that reported by others, shows that the sensitization of animals with pollens is more difficult to accomplish than with proteins from most other sources, and this may explain the unsuccessful attempts of some investigators to demonstrate either antibodies or anaphylactic phenomena. These failures may be due not only to the technic used in sensitizing animals but also to the method

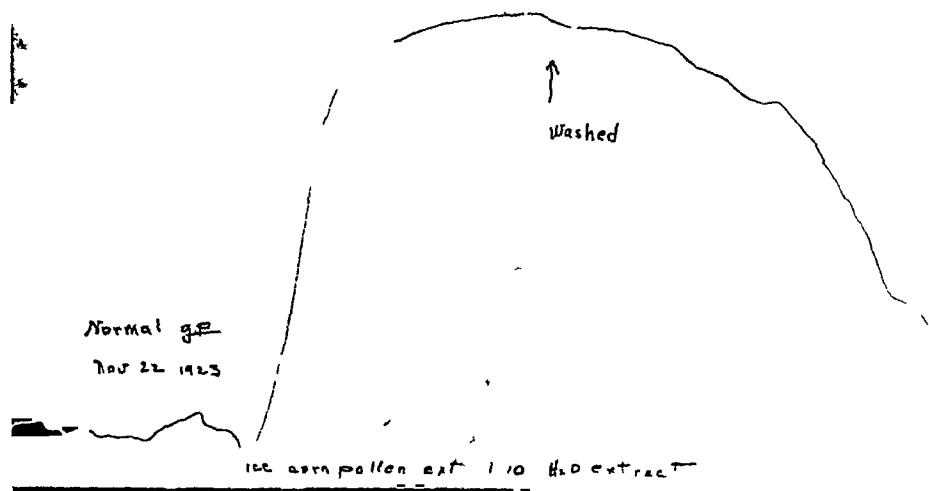


Fig 7—Record of uterine reaction to *Zea mays* (corn) pollen extract in normal guinea-pig

of preparing the extracts which are to be used as antigen. Most of the successful workers except Harrison and Armstrong, who use a glycerol and sodium chloride solution mixture, have used different concentrations of plain or slightly modified solutions of sodium chloride. It is also of great importance to select pollens of known origin and purity as the microscopic examination of specimens obtained from various sources often reveals the presence of different pollens or other contaminations. The indicated specificity of pollens is of great importance clinically, as was again pointed out recently by Beirton, and when generally recognized should make the prophylactic treatment of pollen disease more satisfactory. We recommend that efforts be made to educate the public

to the importance of making accurate observations of the appearance and disappearance of symptoms. These observations, a knowledge of the pollinating season for all local "hay-fever" plants, and information obtained from carefully controlled skin tests make possible a more accurate diagnosis on which to a large extent depends the degree of success of treatment.

At present the evidence for placing pollen disease in the category of anaphylactic phenomena is the following:

- 1 Symptoms produced in sensitive individuals by the inhalation of minute quantities of the specific pollen

- 2 Anaphylactic-like symptoms occasionally produced in sensitive persons by the injection of minute quantities of specific pollen extracts

- 3 Desensitization of sensitive persons by repeated injections of small doses of pollen extracts

- 4 Demonstration of specific antibodies in the blood of man during seasonal attacks

- 5 Frequent association with other disorders of suspected or known anaphylactic nature

- 6 Presence of positive skin and ophthalmic reactions with specific pollens or pollen extracts

- 7 Experimental demonstration of the antigenic property of pollens

- 8 Moderate blood eosinophilia

- 9 Hypotension

CONCLUSIONS

- 1 If suitable procedures are followed the antigenic property of pollens can be demonstrated in specifically sensitized animals by (a) production of characteristic fatal anaphylactic phenomena, (b) positive uterine strip reaction, (c) positive bronchoconstrictions, and (d) production of specific antibodies giving precipitin, complement fixation and passive sensitization reactions

- 2 The response of sensitized uterine strips to *Ambrosia trifida* and *Ambrosia elatior* and to *Helianthus annuus* is specific

- 3 The demonstration of the antigenic property of pollens while not itself proving the anaphylactic nature of pollen disease is highly significant for this interpretation

- 4 *Zea mays* pollen contains an oxytocic principle

THE EFFECT ON PARAMECIA OF BLOOD SERUMS, ESPECIALLY FROM PATIENTS WITH CARCINOMA *

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Many studies on the blood of cancer patients have been made in an effort to determine the presence of a specific substance which is characteristic of the disease and which can be detected soon enough to aid early diagnosis. The tests that have been proposed have involved the assumption that the blood of cancer patients was chemically different from that of normal subjects or of those with other diseases, or that a specific cytolytic enzyme, hemolysin or antibody was present. These tests have proved unsatisfactory either in not being specific for cancer, as has been shown in the study of the phosphorus content of the blood,¹ or as involving so many variable factors as to invalidate them. Underhill and Woodruff² found that an extract of cancer tissue contained a specific substance that was lethal to paramecia. This observation—that there was a substance in cancer tissue different from anything in normal tissue in its effect on living cells—was confirmed by Calkins³ in a study of cancer tissue extract on didinium.

The problem of a specific substance in the blood of cancer patients is still a matter to be investigated. The present work deals with one phase of this problem, experiments having been conducted to determine the presence or absence of a substance in serums from cancer and from other patients which may affect paramecia differently from normal serums. This biologic aspect of the blood in cancer has not received as much attention as the morphologic, physical and chemical phases of the question. Various methods have been devised for studying growth promoting and growth inhibiting substances. Carrell⁴ has shown that leukocyte extracts may accelerate a growth of tissues in vitro. Macht

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* This paper is No. 44 of a series of studies in metabolism from the Harvard Medical School and allied hospitals. The expenses of this investigation have been defrayed, in part, by a grant from the Proctor Fund of the Harvard Medical School for the Study of Chronic Diseases.

1 Buckman, T. E., Minot, G. R., Daland, G. A., and Weld, M. Blood Phosphorus, Its Relation to Cancer and Anemia, *Arch Int Med* **34** 181 (Aug) 1924.

2 Underhill, F. P., and Woodruff, L. L. *J Biol Chem* **15** 401, 1913.

3 Calkins, G. N. *J Cancer Res* **1** 399 (Oct) 1916.

4 Carrell, Alexis. *J Exper Med* **36** 385 (Oct) 1922.

and Lubin⁵ demonstrated that variations of growth of lupin seedlings occurred after serums from patients with various conditions were added to the nutrient mediums. Similar unpublished observations have been made in this laboratory.

Paramecia have been used widely as an index of the growth promoting and destructive action of substances, such as the toxicity of alcohol, the effect of thyroxin and of ultraviolet light. Several investigators have reported work on the influence of serums on paramecia. Takenouchi⁶ found that serums from cold blooded animals were more lethal for paramecia than those taken from warm blooded animals. Ledoux-Lebard⁷ has shown that normal human serums are less toxic to these infusoria than those from animals. He also studied a few serums from sick people and observed that some were more, others less toxic than those from normal subjects.

MATERIAL AND METHODS

Venous blood was withdrawn from the person to be studied, allowed to clot at room temperature and then centrifugalized. The serum was removed to a test tube, kept stoppered to prevent evaporation, and its effect on paramecia was tested on the same day that it was obtained.

The paramecia were kept on hollow ground glass slides in a moist chamber. Daily isolations were made in order to maintain a regular division rate and to observe the condition of the organisms, in accordance with standard methods. Slide cultures from which the stock organisms had been removed three or four days previously were used in the experiments. Thus, the cultures had enough organisms in them with which to work and were approximately of the same concentration of paramecia and of similar bacterial content.

The dilutions of paramecia with serum were made in a blood corpuscle counting pipet so that the proportion of serum and fluid containing paramecia could be controlled. In most of the experiments a dilution of 25 per cent culture and 75 per cent serum was used. This was varied for a study of dilution effects. The culture was drawn up into the pipet and then the serum, the whole amount was blown out on a glass slide, then redrawn into the pipet and expelled a second time. In this way the paramecia were thoroughly mixed with the serum. This drop of material was surrounded by a ring of petrolatum, covered with a glass cover slip and examined under the microscope.

5 Macht, D. I., and Lubin, D. S. *Proc. Soc. Exper. Biol. & Med.* **20** 265, 1922-1923, *J. Pharm. & Exper. Therap.* **22** 413 (Jan.) 1924.

6 Takenouchi, M. *J. Infect. Dis.* **23** 396 (Nov.) 1918.

7 Ledoux-Lebard, M. *Ann. de l'Inst. Pasteur* **16** 510, 1902.

There are many ways of determining the death rate of infusoria, but in this study vesicle formation has been chosen as an index of cytolysis.⁸ This can be observed easily under low power of the microscope and on a number of organisms in the chamber at one time. Vesicle formation can be detected with greater accuracy than the cessation of the cilia beat, or the time at which the paramecia will no longer respond to electrical stimuli. The vesicles are blister-like elevations which, according to Chambers,⁹ appear on the surface of the paramecia when they are placed in an abnormal environment. The vesicles appear to be beneath the pellicle but are external to the protoplasmic surface. Observations were made on from fifteen to forty organisms with each serum tested. The time at which the organisms were mixed with the serum and also the time when the first vesicle appeared on each organism in the chamber were recorded. From this data the percentage of the total number of organisms forming vesicles in a given period of time could be found. The frequency curves of the rate of vesicle formation have been studied in one, three and five minute intervals, as has also the summation of the frequencies.

The five minute interval was chosen for final analysis as it seemed to minimize the differences obtained with normal serums of the same subject from day to day, any errors of observation and manipulation, and the differences due to the daily variation in the resistance of the organisms, except during periods of endomixis.

A pathologic and a normal serum usually were studied on the same day. The comparison of the effects on the same day is especially valuable, even more so than the comparison of results obtained at times quite distant from each other, as differences may be the result of changes in the resistance of the paramecia.

Some twenty-seven observations were made on the serums of nine different normal subjects and also six observations on two of these at the time of menstruation.

Eighteen cases of carcinoma were studied. These included tumors of the breast, palate, rectum, throat, mouth, esophagus and eye. The cases represented many phases of the disease, some were early cases and others were in a terminal, toxic stage. As control observations the serums of patients with diseases other than cancer were studied. These cases included four cases of chronic myelogenous leukemia, two of chronic hemolytic jaundice, five of eclampsia, two of uremia due to chronic nephritis and one case each of the following: chronic lymphatic leukemia, lymphosarcoma, melanotic sarcoma, obstructive jaundice (cause undetermined), toxic jaundice from arsphenamin poisoning, pelvic sepsis, hydroa aestivale, and pernicious anemia in a stage of relapse.

⁸ Bovie, W. T., and Daland, G. A. *Am J Physiol* **66** 55 (Sept.) 1923.

⁹ Chambers, R. *General Cytology*, edited by E. Cowdry, Univ. Chicago Press, 1924, p. 253.

EFFECT OF NORMAL SERUMS ON THE RATE OF VESICLE
FORMATION IN PARAMECIA

The percentage of the total number of organisms forming vesicles in a given frequency interval varies in normal subjects. The height of the frequency curve with each normal serum studied occurred in the frequency interval of from six to ten minutes. For purposes of comparison this interval is used. The mean value for the percentage of the total number of organisms forming vesicles in this period is 63.7 per cent. This figure may vary from 35 to 80 per cent, or approximately ± 25 per cent from the mean. These figures represent the results on different days over a period of several months. From data at hand it seems evident that on a given day the variation between the effects of normal serums on paramecia is not so great.

TABLE 1—*Effect of Blood Serums from Normal Subjects and from Patients with Carcinoma on the Rate of Vesicle Formation in Paramecia*

Frequency Interval in Minutes	Percentage of the Total Number of Paramecia Forming Vesicles in the Given Interval	
	Mean Values for Fourteen Normal Serums, per Cent	Mean Values for Eleven Carcinoma Serums, per Cent
1 to 5	7.6	9.6
6 to 10	63.7	61.9
11 to 15	16.0	18.7
16 to 20	3.2	5.2
21 to 25	3.7	1.1
26 to 30	1.5	0.8
31 to 35	2.0	0.9
36 to 40	1.4	0.0
41 to 45	0.3	0.0
46 to 50	0.3	0.8
51 to 55	0.0	0.8
56 to 60	0.0	
61 to 65	0.3	

The mean values for the percentage of the total number of paramecia forming vesicles in each frequency interval for fourteen comparable tests on six different subjects are given in Table 1.

EFFECT ON PARACEMIA OF BLOOD SERUMS FROM CASES
OF CARCINOMA

The mean values for observations that are comparable for eleven cases of carcinoma are given in Table 1. They are very nearly the same as those in this table for the normal persons. Like normal serums, the lethality of the serums from patients with carcinoma varied. The magnitude of this variation is ± 22 per cent, or approximately of the same degree as that of the normal series. Comparable tests of cancer and normal serums on a given day, not comparable with those made on different days, further indicated no difference in the effect of carcinoma and normal blood serums on paramecia. The carcinoma serum was sometimes slightly more toxic than the normal and sometimes less so. The difference was never striking.

EFFECT ON PARAMECIA OF THE SERUMS FROM OTHER PATHOLOGIC STATES AND DURING MENSTRUATION

Serums from patients other than those with cancer may affect paramecia differently from normal serums. It is to be recalled that comparable tests on different days showed normal serums to cause, at the greatest a ± 25 per cent variation in the rate of vesicle formation and that tests on the same day showed less variation. The serums from some patients caused the percentage of the total number of organisms forming vesicles in the six to ten minute interval to be 50 per cent greater than the normal serums tested on the same day (one case of chronic lymphatic leukemia and one of chronic myelogenous leukemia in remission), and sometimes 50 per cent less (one of melanotic sarcoma, one of eclampsia, and one of obstructive jaundice). Other serums, those from one case each of toxic jaundice, sepsis, pernicious anemia and eclampsia, were from 15 to 25 per cent more toxic than normal serums obtained the same day.

The results obtained in normal women during menstruation are not included as "normal" because it was demonstrated that blood serum during this period was about 20 per cent more toxic for paramecia than at other times. These results are in accord with those of Macht and Lubin⁵ on the effect of serum "menotoxin" on the growth of lupin seedlings and also with that of Herz and Weichbrodt,¹⁰ who have confirmed their work.

EFFECT ON PARAMECIA OF ALTERATIONS OF THE PHYSICAL AND CHEMICAL PROPERTIES OF SERUMS

The differences in the effect on paramecia of blood serums cannot be attributed to a disease per se, but rather to some properties which may or may not be manifestations of disease, much as is the case for the rate of sedimentation of red blood corpuscles. The observations of Takenouchi⁶ and Ledoux-Lebard⁷ also suggest that the differences in the reaction of paramecia to serums from cold and warm blooded animals and from man are due to variations in the physical and chemical composition of the serums.

Further experiments, in which serums have been altered by dilution, presence of hemoglobin, heat and irradiation, indicate that alterations of physical and chemical properties do influence the lethal effect of serums on paramecia.

Effect of Changing Concentration of Serum—It is to be expected that the physical properties of the surrounding medium will be factors in the rate of vesicle formation. Any change in the concentration of

¹⁰ Herz, E., and Weichbrodt, R. Deutsch med Wchnschr **50** 1210 (Sept 5) 1924

the medium will produce a reaction in the organism, the intensity of the reaction varying with conditions. This may be illustrated by observations on the mode and rate of cytolysis with different dilutions of the same serum.

In a concentrated combination of serum and infusion the organism is first stimulated, swimming very rapidly. This is followed by the avoiding reaction, a slowing up process, cessation of motion, vesicle formation and sometimes rupturing, with or without previous vesicle formation.

In a weaker concentration of serum the sequence of the reactions is practically the same. Sometimes, however, instead of forming vesicles, the usual index of cytolysis, the organisms become opaque, this seems to be due to internal disorganization of the protoplasm.

This series of reactions usually takes place in two minutes with 90 per cent serum solution or in about eighty minutes with 25 per cent serum solution. The results with different dilutions of serum are given in Table 2.

TABLE 2—*Effect on Paramecia of Changing the Concentration of the Serum**

Concentration of Serum, per Cent	Frequency Interval in Five Minute Periods (15, 6-10 Minutes)													
	5	10	15	20	25	30	35	40	45	50	55	65	70	80
25	00	00	74	37	74	00	148	148	148	74	111	74	74	37
50	115	192	115	192	115	76	38	38	00	00	38	00	76	00
75	158	630	104	52	52	00	00	00	00	00	00	00	00	00

* The figures in the columns represent the percentage of the total number of organisms forming vesicles in the given frequency interval.

The effect of dilution of normal serums was comparable with that of similarly diluted cancer serums. In some other pathologic conditions the 75 per cent serum solution was more lethal than the same concentration of normal serum and equivalent to a greater concentration (90 per cent) of normal serum. Thus, by varying the concentration of serums the difference between normal and abnormal serums can be clearly shown.

Effect of Presence of Hemoglobin in Serum—It was observed early in this study that serums in which hemoglobin was evident were less toxic to paramecia than serums without hemoglobin. Hence tests recorded above were made with serums free from hemoglobin. Several experiments were done with serums containing hemoglobin liberated mechanically from the red cells. Such a serum was distinctly less toxic than a sample free of hemoglobin from the same subject. A typical experiment is recorded in Table 3.

Effect of Heated Serums—Serum heated in a water bath at 60 degrees C. for ten minutes and then cooled also was found to be

decidedly less toxic than an unheated sample of the same serum Data in Table 3 illustrate this effect

Effect of Irradiated Serums—Alteration in the physical and chemical properties of serums also may be produced by irradiation Roffo and Correa¹¹ have shown that the surface tension and viscosity of the blood may be thus increased

In experiments in this laboratory serum was irradiated in a quartz test tube 70 cm from a Cooper-Hewitt air cooled mercury vapor lamp for a period of one hour The irradiated serum was definitely less toxic than the nonirradiated control A sample experiment is shown in Table 4 The results are in accord with those of similar experiments by Macht and Hill¹² on the effect of irradiated plasma on the growth of lupin seedlings

TABLE 3—*Effect on Paramecia of the Same Serum Before and After Heating and Also After the Liberation of Hemoglobin from the Red Cells into the Serum**

Serum, 75 per Cent Solution	Frequency Interval in Five Minute Periods (15 Minutes, etc.)													
	5	10	15	20	25	30	35	40	50	55	65	70	75	85
Unchanged	91	54.5	18.1	9.1	0.0	0.0	0.0	0.0	0.0	9.1	0.0	0.0	0.0	0.0
Tinged with hemoglobin	0.0	10.3	68.8	10.3	3.4	0.0	3.4	3.4	0.0	0.0	0.0	0.0	0.0	0.0
Previously heated to 60 C for 10 minutes	0.0	8.3	16.6	0.0	16.6	8.3	8.3	0.0	8.3	0.0	8.3	8.3	8.3	8.3

* The figures in the columns represent the percentage of the total number of organisms forming vesicles in the given frequency interval

TABLE 4—*Effect on Paramecia of Blood Serum Irradiated with Ultraviolet Light*

Serum	Frequency Interval in Five Minute Periods (15 Minutes, 6-10 etc.)									
	5	10	15	20	25	30	35	40	45	55
Nonirradiated	3.7	33.3	14.5	11.1	11.1	7.4	14.8	0.0	3.7	0.0
Irradiated 1 hour	0.0	0.0	17.6	11.8	17.6	17.6	29.4	0.0	0.0	5.9

* The serum was irradiated in a quartz test tube, 70 cm from a Cooper-Hewitt air cooled mercury vapor lamp for one hour The figures in the columns give the percentage of the total number of organisms forming vesicles in a given frequency interval

The serums from three patients with carcinoma were studied before and immediately after roentgen-ray treatment with the high voltage, constant potential, short wave length (0.185 angstrom units) apparatus described by Duane¹³ (the dose in these cases was about 600 electrostatic unit seconds, target distance 80 cm and filter one-half millimeter copper) The difference in the lethal effect of the serums was not marked, but in each case the serum after irradiation was less toxic to the paramecia The results of two experiments are given in Table 5

11 Roffo, A. H., and Correa, J. *Semana med* 27:17 (July 3) 1924

12 Macht, D. I., and Hill, E. *J. General Physiol* 6:671 (July) 1924

13 Duane, William. *Am. J. Roentgenol* 9:781 (Dec.) 1922

Thus, while therapeutic roentgen-ray irradiation causes various changes in the blood, including a decreased toxicity of serum for paramecia, this change is not specific, but is comparable apparently to alterations produced by disease, dilution, presence of hemoglobin, heat, and irradiation with ultraviolet light

CONCLUSIONS

1 Paramecia when mixed with human blood serum form vesicles, the rate of formation being an index of the lethality of the serum

TABLE 5—*Effect on Paramecia of Serums from Patients Before and Directly After Roentgen-Ray Treatment**

		Frequency Interval in Five Minute Periods (15, 6-10, etc)					
	Roentgen Ray	5	10	15	20	25	30
Carcinoma of breast	Before	0 0	48 3	29 7	16 1	6 0	0 0
	After	0 0	27 0	31 5	31 5	4 5	4 5
Carcinoma of larynx	Before	0 0	54 6	18 2	18 2	0 0	9 0
	After	0 0	38 4	15 4	7 7	30 4	7 7

* The patients received roentgen ray treatment with a high voltage, constant potential, short wave length apparatus (dose, 600 electrostatic unit seconds, target distance, 80 cm, and filter, one-half millimeter copper). The figures in the columns represent the percentage of the total number of organisms forming vesicles in the given frequency interval

2 No specific substance was found in the blood serum of patients with carcinoma which affected the rate of vesicle formation in paramecia in any way different from normal serum

3 Serums from diseased persons may resemble those from normal subjects in their effects on paramecia, or their toxicity may be greater or less. Serum obtained at the time of menstruation is slightly more toxic than at other times

4 Serums differing in their effects on paramecia may be made to act similarly by altering their concentration

5 Diluted serums, serums containing hemoglobin or that have been heated, serums irradiated with ultraviolet light, and serums of patients who have just received roentgen-ray therapy are all less toxic for paramecia than the control serums

DIABETES

ELECTROCARDIOGRAPHIC STUDIES^{*}

E P W BLITZSTEN, M D

AND

D L SCHRAM, M D

CHICAGO

For many years it has been known that diabetic patients frequently have marked vascular disturbances, particularly of the lower extremities. Heitz¹ has recently shown that all of fifty-three diabetic patients showed some definite vasomotor changes, such as cold feet, edema or cyanosis, while thirty-five, or 66 per cent of the same group, had beginning arteritis, as shown by oscillometry. Joslin² states that 15.1 per cent of diabetic patients in his series of 3,000 cases died of cardiovascular changes, while 2 per cent of this number developed gangrene. He quotes Buerger's³ statement that diabetic gangrene shows typical lesions of atherosclerosis or arteriosclerosis. "These lesions differ in no way from the lesions of the arteries in arteriosclerotic or senile gangrene, and justify the conclusion that diabetic gangrene is due to an atherosclerotic or arteriosclerotic process." This statement has been generally accepted, but no statement has been found in the literature as to the exact etiology of this arteriosclerosis. Fitz and Murphy⁴ state that "cardiovascular or renal disease, including gangrene, often causes death in diabetics. Unfortunately cardiovascular and renal disease are very common in people past the middle period of life." These authors, however, give no more light on the etiology of these changes.

No definite statements about the pure cardiac changes have been found in the literature, nor have a series of electrocardiograms of diabetic patients been published. The only electrocardiographic work on animals seems to be that of Belehradek and Noyons,⁵ who perfused the excised hearts of frogs with pure anhydrous glucose solutions. Electrocardiographic tracings of these isolated hearts were taken at

*From the Cardiographic Laboratory, the Investigative Medical Service, and the Otto Baer Fund for Clinical Research of the Michael Reese Hospital.

1 Heitz, J. Circulatory Derangement in the Legs in Diabetes, *Medecine* 4 783 (July) 1923.

2 Joslin, E. P. Treatment of Diabetes Mellitus, Ed. 3, Philadelphia, Lea and Febiger, 1923.

3 Buerger, L. The Pathology and Diagnosis of So-Called Diabetic Gangrene, *Arch. Diag.* 8 101 (April) 1915.

4 Fitz, R., and Murphy, W. P. Cause of Death in Diabetes Mellitus, *Am. J. M. Sc.* 168 313 (Sept.) 1924.

5 Belehradek, J. and Noyons, A. K. M. L'electrocardiogramme du cœur perfusé au glucose, *Compt. rend. Soc. de biol.* 88 621 (March 10) 1923.

different times during the perfusion. A preliminary period of heart block (complete arrest) was observed, then there was a change in the action current, as shown by an increased amplitude of the main deflection of the galvanometer string.

Our studies were undertaken to see in what way electrocardiographic tracings of diabetic patients differ from those of normal persons of the same age and sex. Furthermore it seemed desirable to ascertain the influence of changes in the blood sugar content on the electrocardiographic tracings.

METHOD

A series of electrocardiograms were taken on diabetic patients with known blood sugar concentrations. A Cambridge type of electrocardiographic instrument, a modification of the Einthoven string galvanometer, was used. In each case all three leads of the electrocardiogram were taken with the patient lying down. Each tracing was accurately standardized, 1 cm swing of the string on the camera being equivalent to 1 millivolt of current. This was the usual hospital routine. Clinical interpretations were made, and the findings carefully noted.

FINDINGS

In the course of several months it was found that a considerable number of electrocardiograms of diabetic patients showed changes that were more marked than the physical findings of the patients seemed to warrant. Tracings were taken of seventy diabetic patients in the hospital, with known blood sugar concentrations. Of these seventy, twenty-one, or 30 per cent, showed notching of the Q-R-S complex in one or more leads. In no case included in this group did Q-R-S measure 0.1 second or more. For this reason it would be difficult to say that there was a true bundle branch block or other anatomic lesion of the conducting pathways in these cases. In all cases, the P-R interval was within normal limits, 0.2 second or less. In all cases the ventricular rate was over 58 per minute. No cases were included in which the diabetes was accompanied by grave cardiac changes, as auricular fibrillation, flutter, idioventricular rhythm, angina pectoris, marked decompensation or aneurysm.

In this series of seventy diabetic patients, forty had high blood sugar concentrations at the time the tracings were taken. The concentration varied between 210 mg and 460 mg of sugar per hundred cubic centimeters of blood, the average being 282 mg. Twenty patients had blood sugar concentrations between 130 and 190 mg, with an average of 150 mg, while ten showed concentrations below 130 mg.

Notching was more frequent in the curves of patients with high blood sugar concentrations than in those with lower concentrations. No

notching occurred in any of the ten electrocardiograms taken of patients with blood sugar concentrations of less than 120 mg per hundred cubic centimeters of blood. When the sugar concentration varied between 130 and 190 mg only five of the twenty cases, or 25 per cent, showed notching of Q-R-S in any of the three leads. With higher sugar concentrations, 210 mg or more, notching occurred much more frequently in thirteen of forty cases, or in 32 per cent.

The swing of the galvanometer string was higher in cases with high blood sugar concentrations than in those with lower concentrations. When the sugar concentration was 210 mg or higher, the average standardized deflection in all the forty cases was 10.7 mm in Lead I, 9.5 mm in Lead II, and 8.5 mm in Lead III. When these figures were more carefully examined it was found that this group could be subdivided into two groups. In the first group were included all those curves which showed no notching in any of the three leads, and here the average swing in the first lead was 11.6 mm, in the second lead, 10.5 mm, and in the third lead, 9.8 mm. In the second group were included all of the curves which had a notched Q-R-S in any of the leads, and here in Lead I the average main deflection was 8.4 mm, in Lead II, 7.0 mm, and in Lead III, only 4.9 mm. There was a marked increase in the notching in Lead III as compared with Lead I.

In the group of twenty diabetic patients with lower blood sugar concentrations, *i. e.*, between 130 and 190 mg of sugar, the average being 150 mg, the average swing for all twenty curves was 11 mm in Lead I, 8.8 mm in Lead II, and 8.3 mm in Lead III. When only those curves were included in which there was no notching in any of the three leads, the average main deflection in Lead I measured 11 mm, in Lead II, 7.2 mm, and in Lead III, 7.5 mm. When only those cases were included in which notched curves were included, the average main deflection in Lead I was 10.1 mm, in Lead II, 8.6 mm, and in Lead III, 5.2 mm.

In the group of ten diabetic patients with blood sugar concentrations below 120 mg, none of the curves showed any evidences of notching. The average main deflection in Lead I was 8.3 mm, in Lead II, 8.3 mm, and Lead III, 8.8 mm.

CONTROL TRACINGS

Control tracings were made of two different groups. The first group consisted of thirty normal men and women approximately the same age as the diabetic patients. These were selected from the hospital personnel and were all persons in good physical condition. The second group was selected from among the patients in the hospital who had approximately the same physical findings as the diabetic patients, excluding the diabetes, *i. e.*, nephritis, myocarditis, hypertension, arteriosclerosis and the like. In the first group, the normal controls, four

curves were notched, but only in the third lead, none showing notching in Leads I or II. The general average for all curves was 7.6 mm in Lead I, in Lead II, 8.4 mm, in Lead III, 5.9 mm. For all curves, excluding notched ones, the average swing in Lead I was 7.3 mm, in Lead II, 8.4 mm, and in Lead III, 6.4 mm. For the four curves which showed notching in Lead III, the average for the first lead was 8.7 mm, for the second lead, 8.5 mm, and for the third lead, 4.7 mm.

In the second group of controls three of the twenty, or 15 per cent, showed definite notching in one or more leads. The average main deflection for all curves was in Lead I, 7 mm, in Lead II, 8.2 mm, and in Lead III, 5.7 mm. When curves that showed notching in any of the three leads were excluded, the main deflection in Lead I measured 8.1 mm, in Lead II, 8.8 mm, and in Lead III, 6.5 mm. When only those curves were included which showed definite notching, the main deflection in Lead I measured 7 mm, in Lead II, 5.6 mm, and

TABLE 1—Average Deflections in Diabetic and Control Cases

Sugar Concentration	R, Lead I	R, Lead II	R, Lead III	Remarks
210 mg or more	10.7 mm	9.5 mm	8.5 mm	All cases (40 cases)
	11.6 mm	10.5 mm	9.8 mm	No notching (27 cases)
	8.4 mm	7.0 mm	4.9 mm	All notched (13 cases)
130 to 190 mg	11.0 mm	8.8 mm	8.3 mm	All cases (20 cases)
	11.0 mm	7.2 mm	7.5 mm	No notching (15 cases)
	10.1 mm	8.6 mm	5.2 mm	All notched (5 cases)
Under 130 mg	8.3 mm	8.3 mm	8.8 mm	All cases, none notched (10 cases)
Normal controls	7.6 mm	8.4 mm	5.9 mm	All cases (30 cases)
	7.3 mm	8.4 mm	6.4 mm	No notching (26 cases)
	8.7 mm	8.5 mm	4.7 mm	All notched in Lead III (4 cases)
Control	7.0 mm	8.2 mm	5.7 mm	All cases (20 cases)
	8.1 mm	8.8 mm	6.5 mm	No notching (17 cases)
	7.0 mm	5.6 mm	3.0 mm	All notched (3 cases)
All controls	7.3 mm	8.3 mm	5.8 mm	All cases (50 control cases)

in Lead III, only 3 mm. The general averages for all control curves were in Lead I, 7.5 mm, in Lead II, 8.3 mm, and in Lead III, 5.8 mm. When all curves that showed notching in any of the three leads were excluded, the main deflection in Lead I measured 7.6 mm, in Lead II, 8.3 mm, and in Lead III, 6.4 mm. In the case of the curves that showed notching in one or more leads, Lead I measured 8 mm, Lead II, 7.2 mm, and Lead III, 3.9 mm.

From Table 1 it is seen that the swing of the galvanometer string was consistently and constantly greater in all groups of diabetic patients than in any or all control groups.

Since diabetic patients with high sugar concentrations showed a greater deflection of the galvanometer string than those with lower concentrations, it was thought advisable to see if changes in the blood sugar concentration would produce changes in the electrocardiographic tracings. For this reason electrocardiographic tracings were taken of a

special group of six diabetic patients throughout the course of treatment in the hospital, when the blood sugar was at different levels. Since there was always considerable variation in the amount of sugar present in the circulating blood at different times of the day, electrocardiographic tracings and blood sugar estimations were made at approximately the same hour each day. The tracings were taken about two hours after breakfast, and two and one half hours after insulin when this was used. As soon as the electrocardiogram was completed, blood was drawn for the sugar estimation. In this way the amount of sugar present at the time the tracing was made was known.

All of the six patients were old diabetic patients, i. e., the disease had been recognized in each case for at least three years. No patient was in a serious condition, none showed evidence of marked acidosis, coma or other complications outside the diabetes. In all cases there was a high initial fasting blood sugar, at least 230 mg of sugar per hundred cubic centimeters of blood. All the patients were kept at a high sugar level for several days, and electrocardiograms and sugar estimations were made daily. This was done in order to get a standard tracing to judge from. At the end of four days, dietetic treatment was begun. The blood sugar dropped gradually. During this period electrocardiograms and blood sugar estimations continued to be made daily. In two cases the blood sugar remained at a high level in spite of dietetic management so that in these two patients insulin was used.

In each case, as the concentration of the blood sugar decreased there was a decrease in the height of the main deflection, except in those cases in which R was notched to begin with. In these cases, R tended to increase in height as the blood sugar decreased, in other words there was a diminution in the notching. The change in height of Q-R-S was fairly rapid, and paralleled the sugar concentration quite closely. When the blood sugar concentration was high, over 300 mg of sugar per hundred cubic centimeters of blood, at the onset of the work, it required at least a week of low blood sugar, 160 mg or less, before Q-R-S remained at a consistently low level. This low level was not permanent, for the height of the main deflection increased gradually with an increase in the sugar content. If the blood sugar concentration was again reduced, the fall to the original low level occurred in a much shorter time than in the beginning (Table 2).

In all of the cases with very high blood sugar concentrations, there was notching of the main deflection. Table 3 is illustrative of the findings in this group.

The same results were obtained in these six cases as in the previous cases, namely, the higher the sugar concentration of the blood at the time the tracing was taken, the higher the main deflection.

ANIMAL STUDIES

This work on human diabetic patients was corroborated by animal experimentation. Dogs were used, and the blood sugar concentration was increased by various means. Electrocardiograms were taken at regular intervals and carefully standardized. In each case, an electrocardiogram was taken as soon as the animal was quiet, and blood was immediately drawn from the jugular vein for sugar estimation. The animal was then anesthetized with ether, and an electrocardiogram taken as soon as relaxation was complete. Blood was drawn from the right jugular vein as soon as the last lead had been taken. It is a well known fact that the percentage of blood sugar rises during anesthesia. The

TABLE 2—*Changes in Deflection Associated with Changes in Blood Sugar Concentration in Same Subject*

Case*	Date	Sugar	R, Lead I	R, Lead II	R, Lead III	Tracing
1	2/10/25	262 mg	13 mm	8 mm	10 mm	1
	2/16/25	190 mg	11 mm	7 mm	9 mm	3
2	2/10/25	242 mg	8 mm	9 mm	5 mm	2
	2/14/25	320 mg	9 mm	9 mm	6 mm	5
	2/18/25	177 mg	8 mm	8 mm	5 mm	8
	2/22/25	230 mg	10 mm	7 mm	5 mm	9

* Case 1, a man, aged 61, Case 2, a man, aged 53

TABLE 3—*Notching of Main Deflection Associated with High Blood Sugar Concentration*

Date	Sugar	R, Lead I	R, Lead II	R, Lead III	Tracing
2/11/25	308 mg	14 mm	4 mm (notched)	2 mm (notched)	1
2/16/25	330 mg	11 mm	5 mm (notched)	2 mm (notched)	5
2/22/25	252 mg	8 mm	4 mm (notched)	2 mm (notched)	6

* The patient, a young man, aged 17, had been under observation for three years, and received 60 units of insulin daily.

work of Banting and Best and their co-workers⁶ corroborates this statement. The latter found that the rise of blood sugar in rabbits is rapid and persists as long as etherization continues. The same findings held good in our work on dogs. There was a marked initial rise of blood sugar, on an average from 92 to 150 mg of sugar per hundred cubic centimeters within twenty minutes after anesthetization, then a continual fairly steady rise for the five or six hours that the experiment was continued. There was a marked initial rise of the main deflection of the string with the beginning of anesthetization, but subsequent tracings showed only slight alteration.

6 Banting, F. G., Best, C. H., Collip, J. B., MacLeod, J. J. R., and Noble, E. C. The Effects of Insulin on Experimental Hyperglycemia, *Am. J. Physiol.* **62**: 559 (Nov.) 1922.

A second group of animals received 500 cc of a 10 per cent solution of glucose intravenously into the right femoral vein. The dogs were kept under the influence of ether throughout the course of the experiment and electrocardiographic tracings and blood sugar estimations were made every hour. In this group (Table 4) as in the preceding, there was a sudden sharp rise of the Q-R-S deflections as soon as the animal was anesthetized. There was a marked increase in the blood sugar concentration as the intravenous solution was continued. There was a slight increase in the swing of the galvanometer string, but this was not as great as had been expected.

A third series of animals received Ringer's solution intravenously in the place of glucose solution. In this group there was the initial increase in height of the main deflection, with etherization, then a slight fall in one case, or constant findings in spite of the fact that there was an increase in the sugar content.

TABLE 4—*Protocols of Two Typical Experiments on Dogs, Illustrating Changes Associated with Increased Blood Sugar Content Due to Ether Anesthesia and Following Intravenous Glucose Injection*

Animal	Blood Sugar	R, Lead I	R, Lead II	R, Lead III	
1	77 mg	8 mm	22 mm	18 mm	Before anesthesia
	130 mg	9 mm	21.5 mm	18.5 mm	After anesthesia
	592 mg	5 mm (notched)	23 mm	21 mm	After 25 gm of glucose
	680 mg	6.5 mm (notched)	24 mm	25 mm	After 45 gm of glucose
2	97 mg	14 mm	13 mm	15 mm	Before anesthesia
	180 mg	15 mm	19 mm	8 mm (slur)	After anesthesia
	294 mg	17 mm	11 mm	6 mm (notched)	After 25 gm of glucose
	480 mg	14 mm	20 mm	10 mm (notched)	After 45 gm of glucose

The last group of animals received insulin following the initial tracings under normal conditions and after preliminary etherization. It was very difficult to overcome the hyperglycemia due to ether anesthesia. As Banting, Best and their co-workers⁷ have shown, there is a latent period during anesthesia before insulin acts. When less than 1 unit of insulin was given intravenously every fifteen minutes, the blood sugar at the end of three hours remained higher than before ether was begun. When more than this amount was used the animal died within an hour or so. These findings are in accord with those of Bliss.⁸ He states that a dog weighing 9 kg. can stand six units of insulin daily, the entire amount within a few hours producing a stormy result. When one unit every four hours was given, fairly good results were obtained by him.

7 Banting, F. G., Best, C. H., Collip, J. B., MacLeod, J. J. R., and Noble, E. C. Effects of Pancreatic Extract (Insulin) on Normal Rabbits, *Am. J. Physiol.* **62**: 162 (Sept.) 1922.

8 Bliss, S. W. The Effects of Insulin on Diabetic Dogs, *J. Metab. Res.* **2**: 385 (Sept.) 1922.

COMMENT

Electrocardiographic tracings of diabetic patients showed notching of the main deflections more often than tracings of other subjects of the same age and sex. The higher the blood sugar concentration, the more frequent the notching, and as the sugar concentration decreased the notching tended to disappear. This notching was not due to a bundle branch lesion because such cases have, according to Lewis,⁹ the following features in common:

1 The amplitude of the chief deflections are more than normal. 2 The initial phases have an unusual duration. 3 The final deflection is opposite in direction to the chief initial deflection, R or S, as the case may be.

None of the tracings included showed any of the three features mentioned above. As yet we are uncertain in what pathologic conditions notching of R occurs with no delay, but it is generally believed that myocardial changes are present in these cases. Since there was a reduction in the notching with decreased sugar content, it seemed probable that there was an improvement of the myocardial condition. It required several weeks of low sugar concentration to cause any marked change in the notching.

The height of the main deflection of the electrocardiographic tracing was greater in diabetic patients than in persons in the control groups of the same age and sex. As the sugar content of the circulating blood increased, there was an increase in the height of Q-R-S in the electrocardiograms, while a decrease in the sugar content caused a smaller swing of the galvanometer string. The sugar content of the circulating blood seemed to influence the action current of the cardiac muscle. These changes can be measured quantitatively, and seem to parallel the quantity of blood sugar fairly exactly. This is in accord with the work of Belehradek and Noyons,⁵ but in our patients the changes were less extreme than in their work on perfused animal hearts. This was to be expected because changes in sugar concentration in human blood can never be so sudden or so marked as in perfused animal hearts. In the case of human hearts, outside factors could not be regulated nor excluded as compared with the regulation of perfused hearts. Within the limit of experimental error, it seemed certain that some change occurred in the electrocardiographic tracing with changes of sugar concentration.

It was not only Q-R-S that showed changes in amplitude with changes in blood sugar concentration. The P wave (due to auricular activity) also showed definite changes in height of excursion. This

⁹ Lewis, T. *The Mechanism and Graphic Registration of the Heart Beat*, London, Shaw and Sons, 1920, p. 120.

was much more difficult to measure quantitatively under the present conditions of standardization. From the very insufficient evidence at hand, it seemed that P changed in the same way as the main deflection changed with blood sugar changes.

Following ether anesthetization, dogs showed a marked and fairly constant hyperglycemia of about the same severity as rabbits show under the same circumstances. The electrocardiograms showed an increase in the height of the main deflection as soon as the dog was under the influence of the ether and relaxation was complete. This rise remained fairly constant as long as the ether was continued, and during this period other factors had little influence on the resulting tracing. When intravenous sugar solutions were given for several hours, there was very little change on the swing of the galvanometer string, after the initial rise due to etherization. Beyond a certain sugar concentration no change could be detected, even when many times the amount of sugar was given which was originally present. When physiologic sodium chlorid solution was given, it was never possible to give enough fluid to overcome the increased blood sugar due to ether. When insulin was used, there was likewise no change because enough insulin to overcome the effects of etherization always caused death within a short time.

Since changes in blood sugar concentration seemed to produce definite changes in the action current, it is suggested that the sugar acts directly on the cardiac muscle. This was more marked when perfused hearts were employed than when human hearts were used. The changes that occurred were more marked when the solution contained a large amount of sugar, but this was not a definitely quantitative thing. The results obtained make it appear possible that sugar in high concentrations may be considered a protoplasmic poison, and acts in a toxic manner on the cardiac muscle. As yet sufficient evidence is lacking for a positive statement on this point.

SUMMARY

Notching of the main deflection of the electrocardiographic curves occurred more frequently in curves of diabetic patients than in curves of control patients.

Changes in the blood sugar concentration were associated with changes in the main deflections of the electrocardiographic tracings.

Increasing the amount of blood sugar in dogs by etherization or by glucose injections caused an increase in the height of the Q-R-S wave, while a decrease in the amount of blood sugar produced a decrease in the amplitude of the Q-R-S wave.

NONSPECIFIC DESENSITIZATION THERAPY IN ALLERGIC ASTHMA

THE EOSINOPHILIC INDEX AS A GUIDE TO INTRAMUSCULAR INJECTION OF VENOM PROTEIN, WITH CASE REPORT

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Nonspecific desensitization and nonspecific protein therapy have become recognized factors in immunity. Rosenau and Anderson¹ have shown that animals receiving repeated injections of foreign proteins become markedly insusceptible to anaphylactic shock. Longcope² writes "It seems impossible to avoid the conclusion that in animals sensitized to two proteins anaphylactic shock to the one reduces temporarily the sensitiveness to the second." Dale³ found that a guinea-pig sensitized to several serums and also to egg white could be desensitized to the serum with egg white. Kolmer,⁴ in referring to nonspecific protein therapy, says that "nonspecific activities of bacterial vaccine may exert beneficial and curative effects in disease to which the bacteria incorporated in the vaccine has no etiologic relationship."

CLINICAL REPORTS ON NONSPECIFIC DESENSITIZATION IN ASTHMA

Numerous clinical reports are accumulating which show the beneficial effect of various nonspecific desensitizing agents in asthma as well as in hay-fever, and other sensitization conditions. Miller⁵ gave intravenous injections of typhoid vaccine in a number of cases of bacterial asthma with relief to some of the patients. Those not benefited were treated with autogenous vaccine. He says "In no case, however, that had failed to be relieved by the typhoid vaccine was there any benefit from autogenous vaccine." Auld⁶ has used peptone intravenously in the treatment of asthma. Recently, he has reported on the beneficial use of peptone and serum in eleven cases. Miller,⁷ in a recent article, concludes "Peptone therapy in asthma and allied conditions offers a simple and effective mode of treatment." Veitch⁸ treated twenty-four

1 Rosenau and Anderson, quoted by Miller, J L. *Am J M Sc* **168** 639 (Nov) 1924

2 Longcope, W T. *Physiological Rev* **3** 290, 1923

3 Dale, H H. *Brit M J* **2** 689 (Oct 29) 1921, *ibid* **1** 45 (Jan 12) 1922

4 Kolmer, J A. *Infection, Immunity and Biological Therapy*, Ed 3, Philadelphia, W B Saunders Company

5 Miller (Footnote 1)

6 Auld, A G. *Brit M J* **1** 696 (May 14) 1921, *ibid*, March 7, 1925

7 Miller, H P. *Illinois M J* **45** 269 (April) 1924

8 Veitch, John. *Brit M J* **1** 13 (Jan 5) 1924

asthmatic patients with weekly intramuscular injections of peptone combined with a mixed catarrheal vaccine. He reports fourteen patients cured, four greatly improved, five improved to a less degree, and one not improved. Van Leeuwen and Varekamp⁹ treated twenty-eight patients with bacterial bronchial asthma who gave positive Pirquet and negative skin tests, with Koch's tuberculin. With this form of non-specific desensitization they report 68 per cent of the patients completely relieved, 10 per cent greatly benefited, 18 per cent decidedly improved and 4 per cent not benefited. The parenteral injection of milk in asthma has been reported of benefit by Schiff¹⁰ and other clinicians.

In a recent article,¹¹ I reported beneficial results in sensitization diseases, including asthma, hay-fever, migraine and epilepsy from the intramuscular injection of venom protein (crotalin) solution, which contains a peptone and a globulin.

EOSINOPHILIA IN ASTHMA

The local collection of eosinophil cells in the lungs in asthma is a clinical observation that has been frequently recorded in textbooks. The almost invariable finding of an eosinophilia in the peripheral blood at some stage of an asthmatic attack, provided the differential leukocyte counts are made at repeated intervals, is very suggestive that the underlying factor, in all cases of true bronchial asthma, is of allergic origin. This increase of eosinophil cells demonstrable before, during or soon after an asthmatic paroxysm is highly significant of the relation of asthmatic hypersensitivity to anaphylactic or anaphylactoid phenomena. The asthmagenic substance to which a patient is sensitized is undoubtedly positively chemotactic for eosinophil cells. Van Leeuwen and Leydner¹² report isolating a muscle stimulating substance from the blood of patients with asthma, urticaria, migraine and epilepsy, all diseases in which an increase of eosinophil cells has been noted. They could not demonstrate its presence in other diseases or in normal subjects. They believe that this toxic substance is related to the "allergic disposition" of the patient.

EOSINOPHILIA IN THE ANAPHYLACTIC-LIKE REACTION FOLLOWING VENOM PROTEIN INJECTION

Investigators are pointing out that commonly an increase in the percentage of eosinophil elements in the differential blood count is pres-

9 Van Leeuwen, W. S., and Varekamp, H. *Lancet* **1** 201, 1921, *ibid* **2** 1366 (Dec 31) 1921.

10 Schiff, N. *Am J M Sc* **166** 664 (Nov) 1923.

11 Spangler, R. H. *M J & Rec* **122** 313 (Sept 16) 1925.

12 Van Leeuwen, W. S., and Leydner. *Brit J Exper Path* **3** 282 (Dec) 1922.

ent during the anaphylactic-like phenomena that follow the parenteral injection of foreign protein substances

In a number of articles ¹³ published since 1912, I have called attention to the fact that the mechanism of the reaction following the intramuscular injection of venom protein (crotalin) solution includes an increase in the percentage of eosinophil cells in the differential count, this is in addition to the lengthening of the clotting of the blood, the increasing of the permeability of the vessel walls and the producing of general cell and glandular stimulation, all recognized factors in the mechanism of the nonspecific protein reaction Schlecht's ¹⁴ experiments on guinea-pigs and dogs, showing that with repeated small injections of various foreign protein substances an eosinophilia is produced, confirm my findings

Since eosinophils are found increased in anaphylaxis and anaphylactic-like conditions it would seem plausible that an estimate of their varying percentage would be of value in determining the degree of the nonspecific reaction, when it is used as a therapeutic measure

SUGGESTED METHOD FOR REGULATING STRENGTH OF DOSE AND FREQUENCY OF ADMINISTRATION OF NONSPECIFIC PROTEIN AGENTS

It would be of the greatest value if a method could be established for regulating the strength of dose and the frequency with which a protein injection should be given in order to keep a patient nonspecifically desensitized The biologic test of giving an injection and depending on the resulting clinical manifestations is most unsatisfactory and often misleading

In an experience with intramuscular injections of venom protein (crotalin) solution covering a period of fifteen years, during which time more than 16,000 intramuscular injections have been given to over 700 patients, I have found that a study of the differential blood count before and after injections with a special reference to the degree of eosinophilia produced (eosinophilic index) offers a satisfactory guide to both strength of dosage and frequency of administration of the protein substance I have reported this method in detail in previous articles,¹⁵ and in submitting it at this time as a practical guide for the administration of nonspecific desensitization therapy in asthma I will summarize my findings briefly

13 Spangler, R H New York M J, Oct 4, 1913, *ibid* **104** 534 (Sept 16) 1916, *ibid* **107** 727 (April 20) 1918

14 Schlecht Deutsch Arch f klin Med **108** 405, 1912

15 Spangler, R H M J & Rec **120** 495 (Nov 19) 1924, Atlantic M J **28** 138 (Dec) 1924

EOSINOPHILIC INDEX A GUIDE

As a rule the highest rise in the percentage of eosinophils, following venom protein injections, which I have used in the range of doses $\frac{1}{400}$ to $\frac{1}{50}$ grain, occurs by the second or third day. In from five to seven days after injection, the eosinophil cells will, as a rule, have dropped to 4 per cent or under (unless a higher percentage range of eosinophils had been present in the patient before the first injection was given), and the patient may be given another injection. It has been my practice not to repeat an injection if the percentage of eosinophil cells by the fifth day has not dropped to within the normal range, or at least as low as they were before starting the protein injections. Moreover, it has not seemed wise to increase the strength of the dose if any given strength is producing an increase of from 8 to 10 per cent of eosinophils by the second or third day after an injection.

The following case report shows the clinical results of nonspecific protein desensitization following repeated intramuscular injections of venom protein (crotalin) solution in a case of bronchial asthma and hay-fever. The accompanying table and chart illustrate the value and significance of recording the eosinophilic index.

REPORT OF CASE

A woman, aged 30, a librarian, who had been suffering with severe asthmatic paroxysms for three years, came under my care, April 11, 1924. Her mother had been subject to nocturnal convulsions at irregular intervals from her eighteenth to her twenty-fifth year. At that age the convulsions stopped and she developed migraine seizures with vomiting, which occurred periodically and persisted until the menopause. A maternal aunt also had migraine.

The patient was the youngest of four children, had been a full term baby and had had a normal birth. She was bottle fed and suffered much with digestive disturbances before teething. As a girl she had "bilious attacks" and frequently suffered with hives. At the twelfth year she began to have migraine, with vomiting. Menstruation was established at 13 years, she was always regular and had little pain. Two months later, in August, 1908, she developed hay-fever, which has recurred periodically each autumn since (sixteen years) in spite of pollen therapy, change of climate, rhinologic treatments, the use of calcium, parathyroid and various other drugs.

Just before the annual hay-fever season of 1921 she developed acute asthmatic paroxysms, at first these were mostly nocturnal but during the year before I saw her they were occurring both day and night, and the patient required epinephrin or morphin hypodermically almost daily. It was necessary for the patient to give up her library work. Food skin tests were negative. Skin reactions to feathers and house dust were positive but the avoidance or removal of these irritants to the best of her ability gave no relief. The physical signs in the case were typical of an acute bronchial asthmatic paroxysm. A urine examination was negative. The blood Wassermann test was negative.

April 11, the hemoglobin was 78 per cent, the red cells 3,950,000, and the white cells 7,200. The differential counts on four days out of the next five during which time she had five severe and four medium asthmatic paroxysms, showed respectively a 9-, 15-, 11- and 14 per cent of eosinophil cells.

April 16, a $\frac{1}{600}$ grain of crotalin was given intramuscularly, followed by a very mild local reaction. The percentage of eosinophils two days later was 11, and there were four light asthmatic attacks.

April 21, a second $\frac{1}{600}$ grain of crotalin was given in the other arm. This was followed by a marked local reaction and two days later the eosinophil cells were 21 per cent. She had had two severe paroxysms, April 20. The percentage of eosinophilia, April 25, was 16, August 26, 11.

April 28, the eosinophils were 9 per cent and I gave a third $\frac{1}{600}$ grain injection of crotalin solution, with another marked local reaction. She had two severe paroxysms, April 29, and one in my office, April 30. There had been, however, an interval of nine days between seizures (the longest freedom from attacks for several months). The eosinophils, April 30, were 16 per cent. May 1, there was a hard paroxysm that lasted several hours. The eosinophils that day were 14 per cent.

May 5, the eosinophils were 10 per cent and I gave a $\frac{1}{600}$ grain of crotalin, which was followed by a good local reaction. Two days later, May 7, the eosinophils were 15 per cent. There had again been an interval of six days without an asthmatic paroxysm when, during the night of May 7, a severe attack occurred.

May 12, the eosinophils were 8 per cent in the morning and I gave a $\frac{1}{600}$ injection of crotalin. That night the patient had a very severe and long asthmatic seizure, which lasted nearly four hours. Two days later, May 14, the eosinophils were 13 per cent.

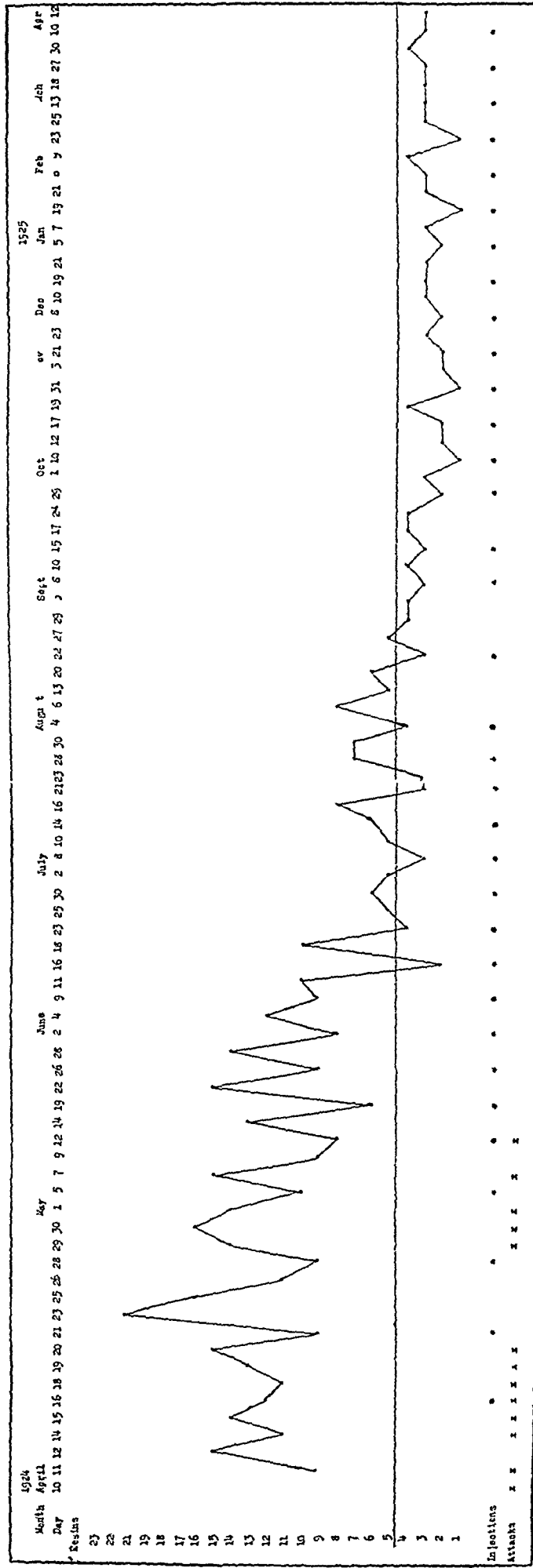
COMMENT ON TABLE AND CHART

By referring to the table and chart, it will be noted that for five days before any protein injections were given and during which time the patient was having repeated acute asthmatic paroxysms, the percentage of eosinophil cells ranged from 9 to 15. The total white count was 6,800. This patient was undoubtedly sensitized to some undetermined asthmogenic substance.

The first crotalin injection, April 16, produced only slight local reaction and no change in the percentage of eosinophils. The second injection, however, five days later (April 21) was followed by a severe local reaction, which lasted forty-eight hours. It was accompanied by a slight leukopenia, the total white count dropped to 4,200. The eosinophil cells, forty-eight hours after the injection, were 21 per cent. There evidently had been superposed a nonspecific sensitization with the production of an anaphylactic-like state as indicated by the leukopenia and the decided increase in the percentage of eosinophil cells. It is of clinical interest that no asthmatic seizures occurred for nine days following this anaphylactic-like phenomenon.

Crotalin injections were then continued at seven day intervals and, as indicated by the accompanying chart and table, were followed each time by an increase of eosinophils, but the percentage range of eosinophils gradually decreased and by June 16, eight weeks after the first crotalin injection, they dropped to within the normal range (4 per cent or under).

During the next two months the percentage of eosinophils showed moderate increase following the crotalin injections, but they never went



Eosinophilic index in a case of allergic asthma nonspecifically desensitized with intramuscular injections of venom protein (crotalin) solution

*Data in Case of Woman Asthmatic Patient, Aged 30, Nonspecifically
Desensitized with Intramuscular Injections of Venom
Protein (CrotaIn) Solution*

Date	Dose of Cro- talin, Grains	Local Reaction	Polymor- phous, per Cent	Differential Leukocyte Count					Attacks
				Small Lympho- cytes	Large Lympho- cytes	Eosino- phils	Baso- phils	Transi- tionals	
1924									
4/11			60	24	4	9	—	3	1 severe 1 medium
4/12			59	16	9	15	1	—	3 medium
4/14			58	28	2	11	—	1	1 severe, 2 medium, 1 severe
4/15			54	23	3	14	1	5	1 severe 2 medium, 2 mild
4/16	1/600	Slight	50	32	4	12	—	2	1 medium
4/18			52	31	4	11	1	1	4 mild
4/19			51	25	6	13	1	4	1 severe
4/20			54	18	8	15	2	3	2 severe
4/21	1/600	Marked	60	23	5	9	—	3	
4/23			49	19	6	21	1	4	
4/25			54	25	3	16	1	1	
4/26			57	22	5	11	—	5	
4/28	1/600	Marked	51	31	5	9	2	3	
4/29			52	25	4	14	1	4	2 severe
4/30			53	26	2	16	—	3	1 severe
5/ 1			48	24	7	14	2	5	1 severe
5/ 5	1/600	Good	50	30	3	10	1	6	
5/ 7			49	27	4	15	—	5	1 severe
5/ 9			51	29	4	9	1	3	
5/12	1/600	Moderate	52	30	5	8	—	5	1 severe
5/14			49	27	5	13	1	5	
5/19	1/600	Very fair	58	27	7	6	—	2	
5/22			59	14	9	15	1	2	
5/26	1/600	Very fair	51	32	4	9	—	4	
5/28			42	36	6	14	—	2	
6/ 2	1/600	Fair	56	28	3	8	1	4	
6/ 4			50	31	4	12	—	3	
6/ 9	1/600	Fair	45	35	7	8	—	5	
6/11			45	30	8	10	2	5	
6/16	1/600	Fair	52	41	3	2	—	2	
6/18			50	30	6	10	1	3	
6/23	1/600	Slight	59	30	3	4	1	3	
6/25			46	38	6	5	1	4	
6/30	1/600	Slight	45	35	9	6	—	5	
7/ 2			48	34	6	5	1	6	
7/ 8	1/400	Fair	55	30	7	3	—	5	
7/10			50	32	8	5	2	3	
7/14	1/400	Fair	48	35	7	6	1	3	
7/16			43	40	4	8	1	4	
7/21	1/400	Fair	50	38	6	3	—	3	
7/23			48	40	7	3	—	2	
7/28	1/400	Fair	48	35	9	7	—	1	
7/30			45	39	6	7	1	3	
8/ 4	1/400	Fair	48	37	7	4	1	3	
8/ 6			50	31	6	8	2	3	
8/13			48	33	7	5	2	5	
8/20			52	31	5	6	1	5	
8/22	1/400	Slight	56	32	6	3	—	3	
8/27			49	34	8	5	1	3	
8/29	1/400	Slight	48	36	8	4	—	3	
9/ 3			50	34	7	4	1	4	
9/ 8	1/400	Slight	50	38	7	3	—	2	
9/10			52	35	6	4	—	3	
9/15	1/400	Slight	50	35	7	3	1	4	
9/17			45	40	7	4	1	3	
9/24			48	37	6	4	2	3	
9/29	1/400	Slight	54	32	6	2	1	5	
10/ 1			50	36	8	3	—	3	
10/10	1/400	Slight	50	38	5	1	2	4	
10/12			48	40	6	2	1	3	
10/17	1/400	Slight	58	32	7	2	—	6	
10/19			52	31	8	4	—	7	
10/31	1/400	Slight	54	33	9	1	1	2	
11/ 3			50	34	8	2	1	5	
11/21	1/400	Slight	52	36	9	2	1	3	
11/23			50	37	6	3	—	4	
12/ 8	1/400	Slight	52	38	7	2	—	1	
12/10			55	34	5	3	1	2	
12/19	1/200	Fair	58	30	7	3	—	2	
12/21			57	29	9	3	—	2	
1925									
1/ 5	1/200	Fair	55	35	8	2	—	—	
1/ 7			53	37	7	3	—	—	
1/19	1/200	Slight	59	34	5	1	—	1	
1/21			55	32	7	3	1	2	

higher than 8 per cent and as a rule each week dropped to within the normal range. Since Aug 22, 1924, and up to the present time, a period of eight months, during which crotalin injections have been given at two or three week intervals, the percentage of eosinophils, as shown on the chart, has never risen above the normal line.

If my interpretation of the eosinophilic index is correct these facts would indicate immunologically that a state of antianaphylaxis has been maintained and that the patient has remained, as I would term it, nonspecifically desensitized.

CLINICAL RESULT

Clinically, it is very interesting to note that the last asthmatic seizure was the severe paroxysm which the patient had on the night of May 12, 1924. Since that time (eleven months) the patient has remained entirely free from all asthmatic symptoms. Moreover, she did not develop any hay-fever symptoms during August and September, 1924, the first season she had missed for sixteen years. Since Sept 1, 1924, this patient has been regularly at her library work and reports that she is "perfectly well and the enemy is conquered" ¹⁶

*Data in Case of Woman Asthmatic Patient, Aged 30, Nonspecifically
Desensitized with Intramuscular Injections of Venom Protein
(Crotalin) Solution—Continued*

Date	Dose of Cro- talin, Grams	Local Reaction	Polymor- phonu- clears, Per Cent	Differential Leukocyte Count					Attacks
				Small Lympho- cytes	Large Lympho- cytes	Eosino- phils	Baso- phils	Transi- tionals	
2/ 6	1/200	Slight	58	30	8	3	—	1	
2/ 9			58	33	5	4	—	—	
2/23	1/200	Slight	57	32	8	1	—	2	
2/25			55	32	7	3	1	2	
3/13	1/200	Slight	55	37	5	3	—	—	
3/18			58	32	7	3	—	—	
3/27	1/200	Slight	55	32	8	3	—	2	
3/30			53	35	8	4	—	—	
4/10	1/200	Slight	56	32	7	3	—	2	
4/12			51	34	6	3	—	3	

CONCLUSIONS

1 Nonspecific desensitization therapy has become a recognized factor in immunity.

2 Various nonspecific protein agents have been found of clinical value in the treatment of allergic asthma and may at times be of benefit even when specific serums and vaccines have given no relief.

3 The increase of eosinophils in the differential blood count in true bronchial asthma points significantly to the presence of an allergic factor in the affliction.

¹⁶ Since this report was written the 1925 season has passed and the patient has remained free of hay-fever symptoms.

4 The varying percentage of eosinophil cells (the eosinophilic index) has been found a valuable guide in regulating the strength of dose and frequency of administration of venom protein when it has been used as a nonspecific desensitizing agent in allergic asthma

THE NATURE OF SO-CALLED SINO-AURICULAR BLOCK ^{*}

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Sino-auricular block is a term applied to a relatively uncommon arrhythmia in which, in an otherwise regular rhythm, dropped beats occur, both auricular and ventricular beats participating in the intermittence. This definition is not strictly true because sino-auricular block is frequently associated with sinus arrhythmia. The long pause is not always equal to exactly two normal cycles, being sometimes shorter, at other times longer. Occasionally, too, the long cycle is preceded by a quickening of the auricular rate and is followed by cycles that are of somewhat greater duration than the average cycles of the normal rhythm.

For a discussion of the literature on the subject of sino-auricular block, reference may be made to Lewis,¹ and also to the papers of Levine² and Eyster and Evans³. Most of the articles are concerned with reports of clinical cases. In them the view has been generally accepted that the arrhythmia is due to a block between the sino-auricular node and the auricle. This assumption is based on the fact that a block between sinus venosus and auricle can be produced in the cold blooded heart when a ligature is placed below the sinus, and also because the arrangement of beats, which frequently, but not always, takes place at the time the intermittence of the auricle occurs, is very much like that seen in auriculoventricular block when a ventricular beat drops out. Lewis¹ has pointed out, however, that there is no clear anatomic or physiologic basis for the acceptance of this view.

The only experimental work that shows definitely that the arrhythmia that is being discussed may actually occur is that of Eyster and Meek⁴. In one experiment, out of a number, in which the sino-auricular node was separated from the rest of the heart on three sides by ligatures and a clamp, clamping the fourth side produced a condition in which the

^{*}From the cardiographic laboratory of the medical clinic of the Johns Hopkins Hospital and University Medical Department.

1 Lewis, T. *The Mechanism and Graphic Representation of the Heart Beat*, London, Shaw and Sons, 1920.

2 Levine, S. A. Observations on Sino-Auricular Block, *Arch Int Med* **17** 153 (Jan) 1916.

3 Eyster, J. A. E., and Evans, J. S. Sino-Auricular Heart Block, with Report of a Case in Man, *Arch Int Med* **16** 832 (Nov) 1915.

4 Eyster, J. A. E., and Meek, W. J. Experiments on the Origin and Conduction of the Cardiac Impulse, VI, Conduction of the Excitation from the Sino-Auricular Node to the Right Auricle and Auriculo-Ventricular Node, *Arch Int Med* **18** 775 (Dec) 1916.

transmission interval between the sino-auricular node and the auriculo-ventricular node increased progressively until each fifth or sixth impulse failed to reach the auriculoventricular node. This was proved by placing electrodes over the sino-auricular and auriculoventricular nodes, each pair being connected with an electrocardiograph. In two other experiments, by completely isolating the sino-auricular node they were able to obtain complete sino-auricular block, the rhythmic rate of the sino-auricular node being greater than that of the rest of the heart.

These experiments are hardly applicable to the condition as it is seen clinically. In the first place, there is no evidence that clinically, when so-called sino-auricular block occurs, the sinus node is completely surrounded by diseased tissue, incapable of transmitting impulses arising in the sinus. In a large percentage of the cases in which sino-auricular block has been present, the arrhythmia has been transient and has resulted apparently from the administration of digitalis,² in other cases it has been present in apparently healthy individuals, of which one, reported by Eyster and Evans,³ may be cited. However, since sino-auricular block is in most instances dependent on vagal action, as it is frequently associated with other evidences of vagal activity and practically always disappears under atropin, and since it may be produced experimentally by drugs that stimulate the vagus nerves,⁵ it is possible that the block between sinus and auricle may be due to vagal tone, just as auriculoventricular block may be occasioned by vagal stimulation. In other words, whereas in Eyster and Meek's experiments a block between sinus and auricle was induced by clamping, clinically a comparable block may be due to vagal tone. This argument, too, is invalid when one attempts to draw an analogy between the experiments of Eyster and Meek, and the condition as it is seen clinically. It has been shown by Lewis and Drury⁶ and more recently by Drury⁷ that when impulses from one part of the auricle are impeded in their transmission to another by clamping, vagal stimulation *decreases* the block rather than increases it. Whether this is true exactly at the sino-auricular junction is not known. But in the experiments of Eyster and Meek the clamp was placed a few millimeters from the border of the sinus node on auricular tissue. One cannot, then, draw inferences as to the clinical condition from these experiments. All that one can say is that they demonstrate the possibility of the occurrence of clinical sino-auricular

5 Cushny, A. R. The Irregularities of the Mammalian Heart Observed Under Aconitin and on Electrical Stimulation, *Heart* **1** 1, 1909-1910. Eyster, J. A. E., and Meek, W. J. Cardiac Irregularities in Morphine Poisoning in the Dog, *Heart* **4** 59, 1912-1913.

6 Lewis, T., and Drury, A. N. The Effect of Vagal Stimulation on Intra-Auricular Block Produced by Pressure or Cooling, *Heart* **10** 179 (April) 1923.

7 Drury, A. N. Further Observations Upon Intra-Auricular Block Produced by Pressure or Cooling, *Heart* **12** 143, 1925.

block due to a lesion similar to the one they have produced experimentally, but that most clinical cases probably do not fall into this group. Barker and Kinsella⁸ have described sino-auricular block in a dog the subject of experimental streptococcus endocarditis. On histologic examination it was found that the sinus node, practically normal itself, was completely surrounded by diseased tissue. However, they are clear in stating that their observation is not applicable to the explanation of most instances of clinical sino-auricular block.

In former experiments⁹ it has been shown that following the intravenous injection of pituitary extract into normal unanesthetized dogs, there developed an arrhythmia which is similar to the one termed sino-auricular block, dependent on central stimulation of the vagus nerves. As the effect of the pituitary extract wore off, sinus arrhythmia appeared. Since there was no sharp transition between the two types of irregularity, the one merging gradually into the other, it was suggestive that there was an intimate relationship between the two. The sino-auricular block seen in these experiments seemed to be comparable to that which one observes clinically, and an opportunity was offered of investigating this arrhythmia further. By placing electrodes directly over the sino-auricular node, at the same time using one of the usual leads, and recording the activity of the sinus and of the auricle as a whole simultaneously, it appeared possible to determine whether or not the irregularity was due to a block between the sinus and the auricle. If a block actually exists, one should obtain on the same record evidence of sinus activity at the time the auricular beat drops out. On the other hand, if the intermittence is due to a failure of the sinus to generate an impulse, then there should be an absence of activity of both the sinus and the auricle as a whole.

EXPERIMENTAL METHODS

Six experiments were done, only three of which are described below. The other three are discarded because after the chest was opened the injection of pituitary extract had practically no effect except to slow the rate of the heart. In the three that are reported below, medium size dogs were used. They were anesthetized by the subcutaneous injection of morphin (or pantopon), followed within fifteen minutes by the administration of urethan by stomach tube. The exact amounts of the drugs are given in the protocols. In order to minimize the disturbances that might result from the operative procedure, 0.5 per cent procain solution was used locally. A part of the fourth right rib was resected

⁸ Barker, P. S., and Kinsella, R. A. Sino-Auricular Block in a Dog Suffering from Experimental Streptococcus Endocarditis, *Heart* 11 81, 1923.

⁹ Resnik, W. H., and Geiling, E. M. K. The Action of Pituitary Extract on the Heart of the Unanesthetized Dog, *J. Clin. Invest.* 1 217 (Feb.) 1925.

under artificial respiration (intratracheal insufflation), this affording ample exposure of the heart. A small incision was made in the pericardium over the sulcus terminalis, and woolen threads moistened with physiologic sodium chloride solution were attached to the surface of the heart, the upper one just below the cavo-appendicular junction, the lower one a few millimeters below this, both on the sulcus terminalis. These electrodes were situated, then, over the normal pacemaker of the heart¹⁰. The woolen threads were attached to the heart by very fine silk ligatures placed in the epicardium, the area of contact with the surface of the heart being about 2 mm in diameter. The woolen threads were then insulated by leading them through small glass tubes which extended from the surface of the heart (particular care being used to see that these ends of the glass tubes were smooth and rounded) and out through the chest wall. The wound in the chest wall was then closed after the air in the pleural cavity was evacuated, respiration then being carried on automatically, a small stream of oxygen flowing through the intratracheal cannula. The protruding ends of the woolen threads were then inserted into kaolin plugged glass tubes filled with a saturated solution of copper sulphate in which were the ends of the wires leading to one galvanometer, which now recorded the electrical activity of the sino-auricular node. To another galvanometer were connected small German silver electrodes sewn under the skin of the right upper and left lower limbs (Lead II), this galvanometer recording the activity of the auricles as a whole, and, of course, of the ventricles. Both records were taken simultaneously on the same film.

After several records of the normal heart's action were taken, from 0.5 to 1 cc of pituitary extract (Armour's Pituitary Liquid) was injected intravenously and further records were made. Finally, atropin was injected, or the vagus nerves tied, in order to determine the effect of vagal release on the arrhythmias that were produced. After the completion of the experiment, the heart was removed and careful note made of the position of the woolen threads. In addition, the tissue lying beneath these electrodes was removed for histologic examination in order to determine whether they actually lay on the sino-auricular node. It may be stated at once that in none of the experiments was the pacemaker of the heart displaced from its normal site at the head of the sino-auricular node.

RESULTS

EXPERIMENT 1—A dog weighing 30 pounds (13.6 kg) was anesthetized with 90 mg of morphin and 14 gm of urethan. The upper electrode on the surface of the heart was 2 mm below the cavo-appendicular junction, the lower one

¹⁰ Lewis, T., Oppenheimer, B. S., and Oppenheimer, A. The Site of Origin of the Mammalian Heart Beat, the Pacemaker in the Dog, *Heart* **2** 147, 1910-1911.

4 mm below the upper, both lying on the sulcus terminalis. Histologic examination of the tissue lying beneath these threads proved that they were situated on the sino-auricular node. Figure 1 is a photograph of the heart after removal from the body, showing the position of the woolen threads.

Following the injection of 1 c.c. of pituitary extract, there appeared in a number of records an arrhythmia that was typical of so-called sino-auricular block. *In no instance was there any evidence of sinus activity during the long pauses, which were approximately twice the length of the normal cycles.* Figure 2 illustrates the arrhythmia that was seen, the upper curve being a record taken by Lead II, the lower one a record taken from the electrodes placed directly on the sino-auricular node. It is clearly seen that there is a complete absence of demonstrable activity in the sino-auricular node as well as in the auricle as a whole during the long pause. In Table 1 are the measurements of a number of successive cycles in several of the records that were taken in this experiment, the figures in heavy type being those of the long cycles.

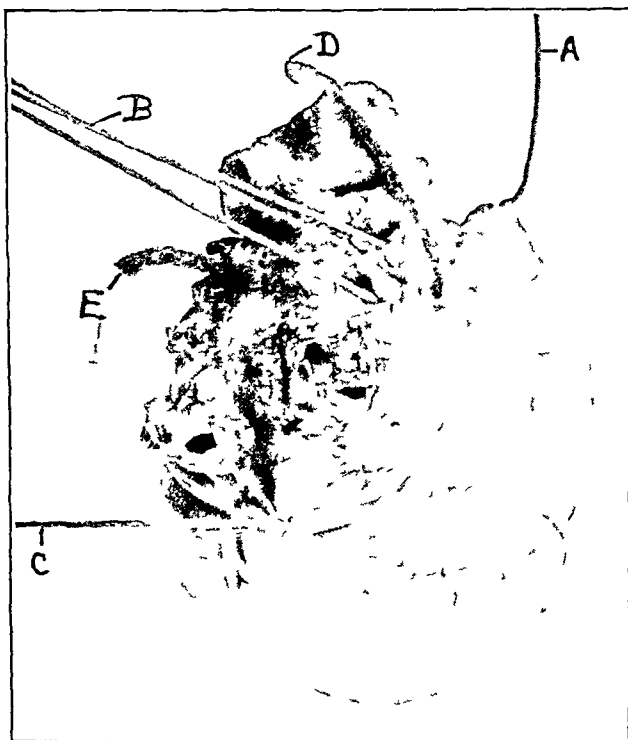


Fig 1—Heart of dog used in Experiment 1 (\times three-fourths, approximately). *A, B and C*, strings attached to tip of right auricle, superior vena cava and inferior vena cava, respectively, *D and E*, upper and lower woolen threads attached to surface of heart, both lying on sulcus terminalis, just below cavo-atrial junction, histologic examination of tissue lying beneath these threads showed that they were situated on sino-auricular node.

In Figure 3 are charted the measurements which are given in Table 1 (except those of Record 12).

This figure demonstrates more clearly perhaps the changes that occurred during the experiment. Record 3 is one taken before the administration of pituitary extract, it shows but slight variation in the length of the cycles. Records 5 and 6 are quite typical of sino-auricular block, the long pauses having about twice the duration of two average short cycles. Record 10, taken when the effect of the pituitary extract was wearing off, is that of an ordinary sinus arrhythmia. Record 11 was taken after the injection of a second cubic centimeter

of pituitary extract The curve of the cycles differs from those of Records 5 and 6, in that following the long pause there is no rapid return to the normal rhythm, instead there is a more gradual slope in the descending limb of the curve Finally, Record 13 shows the complete disappearance of the arrhythmia after vagal paralysis

EXPERIMENT 2—A dog weighing 34 pounds (15.4 kg) was anesthetized with morphin, 48 mg, pantopon, 21 mg, and urethan, 15 gm The upper electrode on the surface of the heart was 2 mm below the cavo-appendicular junction, the lower one 5 mm below the upper one, both lying on the sulcus terminalis Histologic examination of the tissue beneath the electrodes proved that they were resting on the sino-auricular node

TABLE 1—*Measurements of Successive Cycles in Several Records Taken Before and After the Injection of Pituitary Extract in Experiment 1*

Record	Measurements of P-P Intervals *					Remarks
	Seconds					
3	0 52	0 54	0 52	0 51	0 51	Before injection of pituitary extract
	0 51	0 50	0 47	0 46	0 51	
	0 46	0 50	0 49	0 49		
5	0 70	0 70	0 71	1 41	0 65	Records 5-10 taken after 1 cc of pituitary extract
	0 73	0 70	1 36	0 71	0 67	
	0 83	0 62	1 42	0 68	0 70	
6	0 61	0 61	0 68	1 57	0 67	
	0 80	0 62	0 72	1 43	0 66	
	0 68	0 69	0 72	0 68	0 90	
	0 90	0 69	0 72	0 69	1 41	
	0 64	0 62	0 57			
10	0 59	0 53	0 66	0 62	0 73	15 minutes after 1 cc of pituitary extract was injected
	0 71	0 58	0 67			
11	0 61	0 60	0 61	1 39	0 96	Records 11 and 12 taken after a second cubic centimeter of pituitary extract was injected
	0 75	0 86	0 72	0 86	0 73	
	0 76	0 72	0 78	0 68		
12	0 57	0 62	0 60	0 66	1 44	
	0 78	0 89	0 60	1 00	0 75	
	0 73	0 74	0 84	0 72	0 86	
	0 67	0 66	0 64	0 61		
13	0 40	0 40	0 40	0 39	0 39	After the injection of 3 mg of atropin intravenously
	0 39	0 39				

* The figures in this and the subsequent tables are to be read from left to right

The normal records showed a moderate irregularity due to sinus arrhythmia Following the injection of pituitary extract, there appeared in successive records an arrhythmia which is rather difficult to classify The irregularity seemed to be due to a typical sino-auricular block, or to an unusual sinus arrhythmia, or perhaps to a combination of both types of irregularity *In no record, out of the number that were taken, was there any evidence in the electrocardiographic record of activity of the sinus node during the long cycles* In other words, the records showed, as did those of the experiment described above, that the dropping out of the auricular beat during the long pauses is not due to failure of conduction of the impulse from sinus to the auricle, it is due rather to failure of an impulse to arise in the sino-auricular node

In Table 2 are given the measurements of successive cycles in several of the records taken during this experiment These (except Record 12) are charted in Figure 4 Only the first of each group of long cycles is given in heavy type

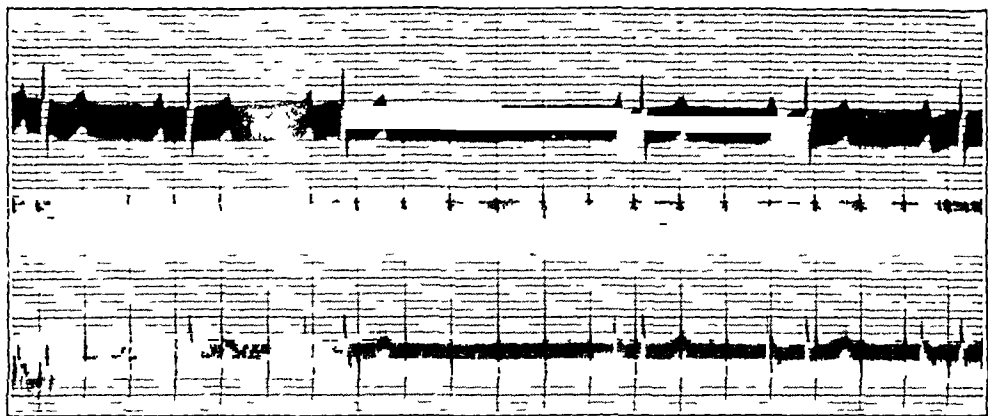


Fig 2—Upper curve, record from Lead II, lower, from electrodes placed directly over upper end of sino-auricular node, records were taken simultaneously on the same film, time lines are 0.2 second apart, upper curve is standardized so that 1 millivolt equals 5 mm, lower so that 1 millivolt equals 4 mm, slight distortion of time lines where two curves merge, probably due to error of adjustment of prisms, vibrations in lower record due to induced currents caused by operation of nearby electric fan

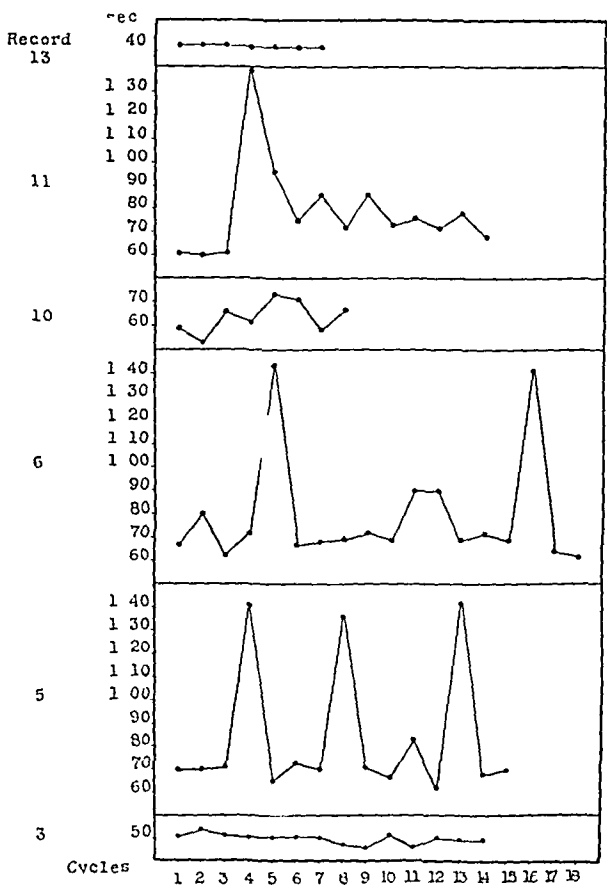


Fig 3—P-P intervals of successive cycles in records in Experiment 1, Record 3, before injection of pituitary extract, Records 5, 6, 10 and 11, after pituitary extract was given, Record 13, after vagus nerves were paralyzed by atropin (Table 1 and text)

The curves of the cycles in the records taken after injection of pituitary extract show many gradations between so-called sino-auricular block and sinus arrhythmia. In most of the curves, there is a steep upstroke, such as one sees in sino-auricular block, Records 3 and 5 are examples. But the onset of the long pause is not always so abrupt. Record 4 is an instance in which the third cycle, just before the long pause, is itself considerably longer than the two preceding cycles. In Record 13 the long cycle is ushered in by two moderately long cycles.

The downstroke of the curves is much more gradual, and it is difficult to judge when the long cycles, ordinarily attributed to sino-auricular block, leave off, and when the normal cycles begin. Record 11 of Figure 3 represents a type intermediate between those shown in Figure 4 and several in Figure 3. The downstroke of the curve is less steep than those of Records 5 and 6 of Figure 3, but it is more abrupt than those of Figure 4.

TABLE 2—Measurements of Successive Cycles in Several Records Taken Before and After the Injection of Pituitary Extract in Experiment 2

Record	Measurements of P P Intervals Seconds					Remarks
2	0.52	0.53	0.51	0.57	0.66	Before injection of pituitary extract
	0.74	0.71	0.69	0.63	0.65	
	0.61	0.61				
3	0.71	0.61	0.53	0.60	1.05	Records 3-13 taken after administration of 1 cc of pituitary extract
	1.03	0.96	0.91	0.90	0.86	
	0.70	0.64	0.55	0.75	1.31	
	1.29	1.04	1.00	0.96		
4	0.66	0.59	0.91	1.34	1.22	
	1.09	1.04	0.95	0.96	0.92	
	0.74	0.64	0.70	1.20	1.36	
	1.22					
5	0.72	0.68	1.38	1.48	1.40	
	1.30	1.17	1.20	1.16	0.99	
	0.70	0.77	0.80	0.78	0.86	
	1.05	1.27				
12	0.68	0.59	0.75	0.78	1.00	
	1.00	0.90	0.77	0.69	0.60	
	0.58					
13	0.61	0.58	0.80	0.71	1.09	
	1.04	0.95	0.84	0.73	0.62	
	0.58	0.71	0.70			
17	0.40	0.40	0.41	0.41	0.41	After administration of 1 mg of atropin
	0.41	0.41	0.41	0.41		

There were numerous dropped ventricular beats in addition to the foregoing irregularities following the administration of pituitary extract. Under the influence of atropin, the rhythm became entirely normal.

EXPERIMENT 3—A dog weighing 22 pounds (10 kg) was anesthetized with pantopon, 64 mg, and urethan, 10 gm. The upper electrode was placed 2 mm below the cavo-appendicular junction, the lower one 6 mm below the upper, both on the sulcus terminalis. Histologic examination of the tissue beneath the electrodes on the surface of the heart revealed sino-auricular nodal tissue.

This experiment may be described briefly. There was a slight irregularity of the rhythm which was accentuated after the administration of pituitary extract, and which disappeared when the left vagus nerve was tied. Table 3 contains the essential data which are illustrated in Figure 5.

Record 6, taken after the giving of pituitary extract, shows in the fourth cycle an abrupt rise, but the long cycle is scarcely long enough to be considered comparable to those of sino-auricular block, it is the record, then, of a definite but not unusually marked sinus arrhythmia. In this record, too, the auricular and sinus activities were synchronous.

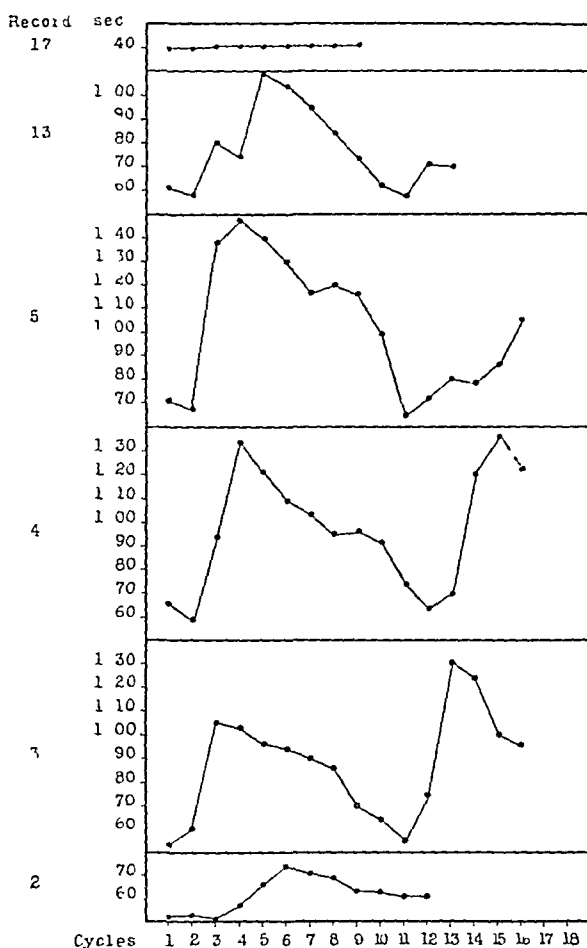


Fig 4—P-P intervals of successive cycles in records in Experiment 2, Record 2, before injection of pituitary extract, Records 3, 4, 5 and 13, after injection of pituitary extract, Record 17, after administration of atropin (Table 2 and text)

TABLE 3—Measurements of Successive Cycles in Three Records Taken During Experiment 3

Record	Measurements of P P Intervals					Remarks
	Seconds					
3	0 35	0 37	0 46	0 46	0 45	Before injection of pituitary extract
	0 47	0 48	0 45	0 39	0 39	
	0 37					
6	0 53	0 52	0 56	0 82	0 62	After injection of 1 cc of pituitary extract
	0 56	0 67	0 53	0 53	0 55	
14	0 31	0 30	0 31	0 30	0 31	After the left vagus nerve was tied
	0 31	0 32	0 31	0 31		

COMMENT

It may be fairly stated that the arrhythmia of Experiment 1 was typical of the one to which the term sino-auricular block has been given, and the arrhythmia seen in Experiment 2 was closely related to so-called sino-auricular block, if not absolutely characteristic. It may also be accepted that these irregularities of rhythm, arising from vagal stimulation, were brought about in a manner essentially identical with that which underlies clinical sino-auricular block. If these assumptions are valid, then the theory of sino-auricular block, as it is applied to the clinical case, is thrown out of court. There is no evidence from the records that I have obtained that during the long cycles, impulses arising in the sinus node are impeded in their transmission to the auricle. The simultaneous records show, rather, that there is a complete cessation of activity of the sinus node, so far as such activity is demonstrable by the electrocardiograph. As I have indicated before, it is possible that clin-

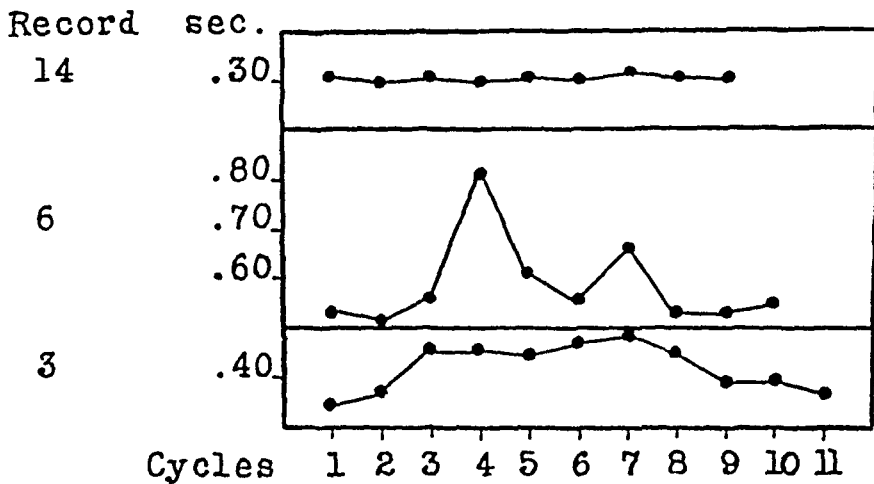


Fig 5—P-P intervals of successive cycles in records in Experiment 3, Record 3, before injection of pituitary extract, Record 6, after injection of pituitary extract, Record 14, after left vagus nerve was tied (Table 3 and text)

ical cases might occur in which there was actually a block between sinus and auricle. Most, if not all, of the reported cases almost certainly do not fall into this group, and for this reason the term sino-auricular block is an incorrect one. Sino-auricular standstill would be a more accurate expression of the clinical condition.

It has been observed before² that so-called sino-auricular block is frequently associated with sinus arrhythmia. The experiments I have reported point rather definitely to the reason for this coincidence. One can detect in a series of curves practically all transitions between typical sino-auricular block and typical sinus arrhythmia. So-called sino-auricular block is really a special form of sinus arrhythmia, and in these experiments, as in most clinical cases, both irregularities are due to the influence of the vagus nerves on impulse formation in the sino-auricular

node While so-called sino-auricular block is usually due to vagal influence, it is not necessarily always brought about by this cause Experimentally, I have observed on one occasion the appearance of this arrhythmia in the late stage of anoxemia, when the heart was completely under the influence of atropin,¹¹ but in this experiment, too, there was definite evidence that impulse formation in the sino-auricular node was being depressed by direct effect of anoxemia on the myocardium

SUMMARY AND CONCLUSIONS

1 When so-called sino-auricular block comparable to that seen clinically is produced experimentally, there is no evidence that a block exists between the sino-auricular node and the auricles Records taken simultaneously from Lead II, and from electrodes placed directly on the sino-auricular node, show a complete absence of demonstrable sinus activity during the long pauses The arrhythmia is due, not to failure of conduction from sinus to auricles, but to failure of an impulse to arise in the sino-auricular node during the long pause

2 Since "sino-auricular block" does not properly designate the arrhythmia to which it has been applied, in most, if not all, clinical cases it is a term that should be dropped "Sino-auricular standstill" is suggested as a more accurate expression of the condition as it is seen clinically

3 Practically complete transitions between sino-auricular standstill and sinus arrhythmia may be obtained in a series of records taken after the injection of pituitary extract This is due to the fact that both irregularities occur as the result of vagal influence on impulse formation in the sino-auricular node Sino-auricular standstill is merely a special form of sinus arrhythmia

11 Resnik, W H Observations on the Effect of Anoxemia on the Heart, III, Changes in the Auricles, with Particular Reference to the Relationship Between Anoxemia and Auricular Fibrillation, to be published

IDENTIFICATION OF THREE TYPES OF MONONUCLEAR PHAGOCYTES IN THE PERIPHERAL BLOOD⁴

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The presence in the peripheral blood of a relatively large number of granular polymorphonuclear leukocytes and of lymphocytes has greatly facilitated the investigations by means of which these white blood cells have been traced to the bone marrow and to the lymphoid tissue, respectively. The determination of the origin of the mononuclear phagocytes which are much less numerous in the normal blood has been attended with greater difficulty. Ehrlich,¹ by the use of differential staining and especially by the method employing the triacid stain, recognized in the blood a large mononuclear leukocyte which he thought came from the spleen and the bone marrow and which was in the circulating blood transformed into the polymorphonuclear leukocyte. He observed further that some mononuclear leukocytes had indentations in the nuclei together with a few fine granules in the cytoplasm, and these he regarded as the transitional forms between the mononuclear leukocyte and the polymorphonuclear neutrophil. The two terms "large mononuclear leukocyte" and "transitional leukocyte" have been retained rather persistently by clinical hematologists although the view held by Ehrlich of their origin has generally been considered incorrect.

Mallory² in 1898 named the mononuclear phagocytes of typhoid lesions "endothelial leukocytes." Since that time cumulative evidence has given much support to the view that the mononuclear phagocytes in the tissues and blood arise from endothelium. Aschoff and Kiyono³ by vital staining with colloidal dyes traced these free cells to the reticulo-endothelium. Sabin,⁴ in the chick embryo, saw endothelial cells detach themselves from the walls of veins and become free in the lumina of the vessels. After marking the vascular endothelium by the intravenous

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1 Ehrlich, P. Ueber die specifischen Granulationen des Blutes, *Arch f Anat u Physiol*, 1879, p. 571.

2 Mallory, F. B. Histological Study of Typhoid Fever, *J Exper Med* 3: 611, 1898.

3 Aschoff, L., and Kiyono, K. Zur Frage der Grossen Nononuklearen, 1913, *Folia Haematol* 15: 383, 1913.

4 Sabin, F. R. Studies on the Origin of Blood Vessels and of Red Blood Corpuscles as Seen in the Living Blastoderm of Chicks During the Second Day of Incubation, Contribution to Embryology 9, Carnegie Inst. Publication 272, p. 213.

injection of carbon in suspension, I⁵ observed the carbon laden cells to undergo mitosis and to separate and assume the same appearance as the free phagocytes

Recently Sabin, Doan and Cunningham⁶ gave the first noteworthy evidence that these phagocytes are not of a single type by demonstrating in peritoneal exudates with supravital stains a cell with the neutral red granules grouped in the *hof* of the nucleus in the form of a rosette in distinction to a second type with neutral red granules scattered diffusely. The final conclusions reached by Cunningham, Sabin and Doan⁷ are that the rosette type of cell is the monocyte and that it arises in common with the granular leukocytes and lymphocytes from a mesenchymal or reticular stem cell and that the clasmatoocytes and the erythroblastic cells arise from endothelium. They were successful in demonstrating the rosette cell in the spleen, bone marrow, omentum and liver but did not identify it in the lymph nodes⁸. I⁹ repeated their experiments on the peritoneal exudates of rabbits and in addition to the two types described by them found a third "hyaline" type with little affinity for neutral red. Examination by me of the lymph nodes, spleen and liver, fresh and in paraffin sections that had the dye mordanted in them, gave evidence which appears to be conclusive that the rosette type of cell first described by Sabin and her co-workers, Doan and Cunningham, arises from the reticulo-endothelium of the lymph nodes. The free cells of this type were seen to undergo mitosis but only in the lymph node have they been demonstrated to arise from fixed tissue.

In rabbits that have received many intraperitoneal injections of whole rabbit's blood evidence of the origin of the rosette cell from the reticulum of the lymph nodes seems beyond controversy. In the enlarged nodes of such rabbits perfect rosettes are present in the large attached endothelial cells. The large rosette cells that are present both in peritoneal exudates and in lymph nodes are essentially abnormal, stimulated forms but such rosettes afford direct evidence of the identity of the two cells. It was observed in rabbits previously injected intravenously with large amounts of India ink that one-half the hyaline

5 McJunkin, F. A. The Origin of the Phagocytic Mononuclear Cells of the Peripheral Blood, *Am J Anat* **25** 27 (Jan) 1919

6 Sabin, F. R., Doan, C. A., and Cunningham, R. S. The Separation of the Phagocytic Cells of the Peritoneal Exudate into Two Distinct Types, *Proc Exper Biol & Med* **31** 330, 1924

7 Cunningham, R. S., Sabin, F. R., and Doan, C. A. The Development of Leukocytes, Lymphocytes and Monocytes from a Specific Stem Cell in Adult Tissues, Contribution to Embryology, Carnegie Inst of Wash Publication 361, p 227

8 Cunningham, Sabin and Doan (Footnote 7, p 259)

9 McJunkin, F. A. The Origin of the Mononuclear Phagocytes of Peritoneal Exudates, *Am J Path* **1** 305 (May) 1925

cells may contain ink particles at a time when less than one in a hundred other mononuclear phagocytes carried ink granules. The Kupffer cells of the liver react to neutral red, to trypan blue and to carbon in suspension after the fashion of the hyaline type. These observations indicate that the "hyaline" cells present in the exudate are derived from vascular endothelium of the type that lines the sinusoids of the liver. The "hyaline" cell, especially when phagocytic, may acquire more of the neutral red, but the phagocyte, abundant in the exudates and characterized by a diffuse neutral red granulation, was thought to arise chiefly through a dispersion of the neutral red granules of the rosette cell with loss of the wreath appearance. At an earlier date I⁹ adopted the Mallory term of "endothelial leukocyte" for the mononuclear phagocytes. It is the only term that names accurately the origin of these phagocytes. Separation of the group into the lymphendotheliocytes of lymph vascular origin and the hemendotheliocytes of blood vascular origin now appears possible, at least under certain experimental conditions.

Sabin, Cunningham and Doan constantly refer to the rosette type of cell as the monocyte of the rabbit's blood and apply to it this term, which Naegeli¹⁰ applied to the granular oxydase reacting mononuclear cell of peripheral human blood. Simpson¹¹ found no mononuclear cells present in the blood of the rabbit which give the oxydase reaction and I⁹ found this to be true for the peritoneal exudate cells so far as the peroxydase reaction with benzidin is concerned. By comparison of the mononuclear cells of human blood that react to benzidin with those that ingest carbon in vitro the conclusion was reached by myself and Charlton¹² in 1918 that the benzidin reacting cell of the peripheral blood was the endothelial phagocyte of the tissue. Subsequently in 1924 I¹³ devised a method by which peroxydase staining was made applicable to paraffin sections of human tissue and found that only small foci of reacting cytoplasm were demonstrable in occasional Kupffer cells and that no cells of the human lymph gland reacted. The irregular reaction seen occasionally in phagocytic endothelium had the appearance of phagocytized material. In the paraffin sections reacting mononuclear phagocytes were seen only in the spleen. This discrepancy between the blood and the tissues as regards peroxydase staining with benzidin was not explained at that time. The present investigation of the blood was therefore undertaken to determine first the occurrence in the blood of

10 Naegeli, O. *Blutkrankheiten und Blutdiagnostic*, Berlin, 1923.

11 Simpson, M. E. *The Experimental Production of Macrophages in the Circulating Blood*, *J. M. Res.* **43** 77 (April-May) 1922.

12 McJunkin, F. A., and Charlton, A. G. *The Practical Identification of Endothelial Leukocytes in Differential Blood Counting*, *Arch. Int. Med.* **22** 157 (Aug.) 1918.

13 McJunkin, F. A. *Peroxydase Staining with Benzidin in Paraffin Sections of Human Tissue*, *Anat. Rec.* **24** 67, 1922.

hemendotheliocytes and the lymphendotheliocytes, which had previously been demonstrated in peritoneal exudates,⁹ and, second, the relationship of these two phagocytes to the mononuclear benzidin reacting phagocytes of human blood

METHODS

*Method A Phagocytosis in Vitro*¹⁴—To 2 c c of 3.8 per cent sodium citrate solution in a graduated centrifuge tube, 3 c c of blood is added. The citrated blood is mixed with 1 drop of India ink (Higgins') and the mixture incubated at 37 C for ten minutes. The mixture is centrifugated at low and then at high speed and the tube, after the careful complete removal of the supernatant liquid, returned to the incubator for fifteen minutes. The leukocytic layer is removed with a capillary pipet and smears are made on slides. It is essential to spread out completely the droplet and to guard against the smear reaching the edge of the slide, otherwise, most of the leukocytes may be lost. A small bulb pipet with the capillary at an angle of 135 degrees is convenient for removal of the liquid from the centrifuge tube. The 3 c c of blood may be obtained from the finger of some person by "milking" after puncturing deeply near the thumb angle of the nail. In the guinea-pig the blood is obtained from the heart. By placing a rabbit head downward held firmly in a suitable box this amount of blood flows readily from the marginal ear vein after insertion and removal of a large needle. The phagocytosis obtained with human blood is quite complete but is very much less satisfactory in the case of the guinea-pig and the rabbit.

*Method B Peroxydase Staining of Smears with Benzidin*¹⁵—The fresh preparation of the leukocytic layer is covered for from thirty to sixty seconds with about 10 drops of an alcoholic solution of benzidin consisting of 100 mg of dry benzidin dissolved in 25 c c of acetone-free 80 per cent methyl alcohol that contains 1 drop of hydrogen peroxid. To the alcoholic solution 10 drops of distilled water is added and two minutes are allowed for the reaction to take place. In the case of guinea-pig or rabbit blood the reaction requires from five to ten minutes. Smears of guinea-pig or rabbit blood must be treated as soon as they become dry but the leukocytes of human blood react after several hours. The solution is washed off and hematoxylin (Harris' without acetic acid) applied for from twenty to sixty seconds. The hematoxylin is followed by 0.01 per cent eosin solution for twenty seconds. The

14 McJunkin, F. A. A Simple Technique for the Demonstration of a Phagocytic Mononuclear Cell in Peripheral Blood, *Arch Int Med* **21** 59 (Jan) 1918.

15 McJunkin, F. A. A Benzidin-Polychrome Stain for Blood, *J A M A* **74** 17 (Jan 3) 1920.

preparation may also be stained by allowing Wright's stain properly diluted to act for from five to ten minutes

*Method C Peroxydase Staining with Benzidin in Paraffin Sections*¹³—The tissue, fixed for one day in 4 per cent formaldehyd solution is cut into small bits not more than 2 mm in thickness, placed in 70 per cent acetone solution for two hours, pure acetone for thirty minutes, benzene for twenty minutes, and 52 C paraffin for twenty minutes. Thin sections are attached to slides by allowing them to dry over night at room temperature. The paraffin is removed with benzene (ten seconds) and acetone (ten seconds). The sections are covered with the diluted benzidin solution for five minutes, washed and stained with hematoxylin and eosin, as in Method B. After the eosin is washed off the excess of water is carefully removed with a soft cloth and the preparation dehydrated with acetone (ten seconds) and benzene (ten seconds), when it is mounted in balsam. To dehydrate in so short a time the solutions are run over the preparations from dropping bottles.

Method D Supravital Staining of Leukocytes with Neutral Red—In a centrifuge tube, to 10 c.c. of a neutral red solution consisting of 19 parts of saline solution and 1 part of saline solution saturated with neutral red (Gruebler) 2 or 3 drops of the fresh leukocytic layer without ink is added. After being mixed and allowed to stand for about ten minutes the cells are sedimented in the centrifuge tube and all of the supernatant liquid except about 2 drops is removed. With a platinum loop the cells are mixed with the liquid and a small droplet is transferred to the center of a cover glass rimmed with petrolatum and at once inverted on a slide for examination with oil immersion lens. The cells are permitted to remain in the citrate the shortest time possible.

*Method E Zenker-Formaldehyd Solution (Formol) Method of Mordanting the Supravital Dye in Smears*⁹—The reaction has usually reached the maximum intensity after about forty-five minutes contact with the dye. Heavy smears are made and just as dying becomes complete they are dropped into a solution consisting of 5 parts of 40 per cent formaldehyd and 95 parts of Zenker's fluid without acetic acid. After two hours' fixation the preparations are washed and at once covered with hematoxylin (Harris' without acetic acid) for two minutes. The hematoxylin is washed off and the slides immediately blotted dry. The dye granules are well preserved but the nuclear staining is faint.

Method F Iodin Vapor Method of Mordanting the Supravital Dye in Smears—Tissue cultures stained supravitaly have been successfully fixed in iodine vapor for microscopic examination and photographic pur-

poses by Lewis¹⁶ The smears of the fresh leukocytic layer, as they become dry, are placed for thirty minutes above iodine crystals in a glass jar 4 inches (10.1 cm) in diameter and 6 inches (15.2 cm) high with a ground glass cover They are removed from the iodine vapor, covered with hematoxylin (Harris' without acetic acid) for one minute, washed and at once blotted dry with filter paper The nuclei are well stained with hematoxylin, but the dye granules are apt to range from brown to black Lewis does not mention using a nuclear stain

*Method G Zenker-Formaldehyd Solution (Formol) Method of Preserving Supravital Stains in Paraffin Sections*⁹—Under anesthesia or immediately after removal from the living body a saline solution saturated with neutral red (Gruebler) is injected into the lymph nodes, spleen or liver until the tissue becomes distended by the liquid A second injection after a few minutes is advisable After a half-hour the tissue is placed for twelve hours in Zenker-formaldehyd solution (formol) consisting of 15 cc of 40 per cent formaldehyd and 85 cc of Zenker's fluid without acetic acid It is then cut into pieces not to exceed 3 mm in thickness and transferred to Zenker's fluid without acetic acid for from twelve to twenty-four hours The bits of tissue are then placed in pure absolute acetone for one hour (two changes), in benzene for twenty minutes and in 52 C paraffin for twenty minutes Extra thin sections are then attached to slides with albumin fixative by allowing to dry over night at room temperature To stain, the paraffin is removed with xylene (ten seconds) and pure acetone (ten seconds) After immersion in water (five seconds) the slide is stained very lightly with hematoxylin (Harris' without acetic acid) for about five seconds The section is then dehydrated with pure acetone for ten seconds, at once covered with xylene for ten seconds and mounted in balsam To limit the action of the acetone and xylene to these times, the slides are stained singly and the solutions run over them from dropping bottles

MONONUCLEAR PHAGOCYTES OF RABBIT'S BLOOD

A normal rabbit, Rabbit H 16 (accompanying table), was one of several full grown rabbits examined In supravital films (Method D) before any injections had been made, there were present 3 per cent lymphendotheliocytes (the count from a fresh preparation) The blood of this rabbit was frequently examined to determine the occurrence in it of a carbon marked mononuclear cell that did not react to neutral red (Method A, followed by Method D), but no such cell was found On this basis it was concluded that hemendotheliocytes were not present

¹⁶ Lewis, W H Endothelium in Tissue Cultures, *Am J Anat* 30 39 1922

Satisfactory phagocytosis of carbon has rarely been obtained in the citrated blood of the rabbit or guinea-pig. Examination of smears (Method B) showed an absence of benzidin reacting mononuclear cells. All mononuclear phagocytes reacting to neutral red were considered to be of the lymphendothelial type. In the fresh films they are about the size of the polymorphonuclear leukocytes with most of the dye in a focus but frequently dye granules are present elsewhere in the cytoplasm. In an occasional cell the dye is in the form of a characteristic

Rabbit H 16, Weight, 2,010 Gm

Date	Treatment	Examination of Blood
4/ 6/25	10 c.c. of blood intraperitoneally (after blood examination)	Lymphendotheliocytes,* 3 per cent, hemendotheliocytes,† none
4/ 7/25	10 c.c. of blood intraperitoneally	
4/ 8/25	40 c.c. of blood intraperitoneally	
4/10/25	4 c.c. of ink intravenously† (after blood examination)	Lymphendotheliocytes, 7.2 per cent, hemendotheliocytes, none
4/13/25	4 c.c. of ink intravenously (after blood examination)	
4/14/25		Lymphendotheliocytes, 4 per cent, hemendotheliocytes, 1 in 15 minutes
4/20/25	4 c.c. of ink intravenously	
4/21/25		Lymphendotheliocytes, 4.2 per cent, hemendotheliocytes, 3 in 15 minutes
4/22/25		Unchanged
4/27/25	4 c.c. of ink intravenously	
4/28/25	4 c.c. of ink (after blood examination)	Lymphendotheliocytes, 3 per cent, hemendotheliocytes, 1 per cent
4/29/25	15 c.c. of blood intraperitoneally	Detailed count of 1,000 cells: granular leukocytes, 59.1 per cent, lymphocytes, 27.9 per cent, lymphendotheliocytes, 3.6 per cent, hemendotheliocytes with carbon, 3.7 per cent, and hemendotheliocytes (?) without carbon, 5.7 per cent‡
4/30/25	20 c.c. of blood intraperitoneally	
5/ 1/25	5 c.c. of ink intravenously	Lymphendotheliocytes, 7 per cent, hemendotheliocytes, 1 per cent, with much carbon
5/ 2/25	20 c.c. of blood intraperitoneally	
5/ 4/25	20 c.c. of blood intraperitoneally	
5/ 5/25	5 c.c. of ink intravenously	Lymphocytes, 20.5 per cent, granular leukocytes, 67.6 per cent, lymphendotheliocytes, 6.8 per cent, hemendotheliocytes, with carbon, 2.2 per cent, hemendotheliocytes (?), without carbon, 2.9 per cent
5/ 6/25	Chloroform	Groin lymph nodes showed a slight tinge of gray and measured 3 by 5 by 9 mm

* Neutral red may or may not be arranged in form of rosette, Method D

† Methods A and D

‡ The ear vein was used

§ Without ingested carbon identification of the hemendotheliocytes is uncertain

rosette. These wreath forms are uncommon in normal human or guinea-pig blood. The wreath form of this cell is apt to be larger than the granular leukocyte while the cell with a dye focus lacking the rosette or wreath appearance is usually slightly smaller. The interpretation of this observation is that the smaller leukocyte is the older one. The lymphocytes may contain no neutral red or they may show from one to several small granules in a focus of cytoplasm. In the lymphendotheliocytes as seen in fixed smears the dye appears as large, heavy dye masses.

Following three daily injections, April 6, 7 and 8, of whole rabbit's blood into the peritoneal cavity of Rabbit H 16, the lymphendotheliocytes numbered 7.2 per cent, April 10 (table). No hemendotheliocytes were present. April 14, the lymphendotheliocytes had dropped to 4 per cent. April 10, 13, 20, 27, 28 and 30, the rabbit received injections of 4 or 5 c.c. of India ink (Higgins') into the ear vein. April 29, the hemendotheliocytes marked by carbon numbered 3.7 per cent. When this number is present there is an impression that these cells contain more carbon than the lymphendotheliocytes, and, since the cytoplasm is devoid of neutral red, the cell is entirely distinctive in supravital preparations. Careful observation is often necessary to outline the hyaline cytoplasm. In a smaller, 1,250 gm. rabbit that received only two injections of 5 c.c. of ink, these cells numbered 5.2 per cent. eighteen hours after the second dose. The usual experience has been that the appearance of these cells in the peripheral blood is quite irregular and follows large doses of the suspensions. April 29, there were present 5.7 per cent. carbon-free mononuclear cells that resembled those with carbon and on incubation with dilute ink some of these appeared to ingest the carbon particles, but the results obtained by this method were not conclusive. The only certain method of identifying these cells in the rabbit is by the intravenous injection of carbon. These hyaline carbon marked hemendotheliocytes vary in size (Fig 1, *A* and *B*). A great majority of those appearing in the peripheral blood have a spherical nucleus and often a relatively abundant cytoplasm.

Examination, March 6, of cells in fixed supravital smears from a normal rabbit, H 16, by Method F revealed lymphocytes, 32.1 per cent, polymorphonuclears, 63.1 per cent, and lymphendotheliocytes, 4.8 per cent. In fresh supravital stains the percentage of lymphendotheliocytes was 3. It has been the usual experience that a larger number of neutral red reacting mononuclear cells can be identified in the fixed preparations. They vary from a size intermediate between lymphocyte and polymorphonuclear leukocyte to a size somewhat larger than the polymorphonuclear. The amount of dye is large (Fig 1, *C* and *D*) and is placed unilateral with a flattened side of the nucleus toward it, or the dye granules may lie in a V shaped depression of the nucleus. In the lymphocytes from one to several minute dye granules appear at one point in the cytoplasm near the nucleus (Fig 1, *C*). The larger of the lymphocytes may reach the size of the small lymphendotheliocytes. In the cells that present a neutral red rosette in the fresh specimen there is a central vacuole in a fixed preparation (Fig 1, *C*). The neutral red granules in the polymorphonuclear leukocytes are small but distinct (Fig 1, *D*). It is a noteworthy fact that there is an occasional polynucleated cell that contains much neutral red and that in smears treated with benzidin there is an occasional

nongranular polymorphonuclear leukocyte that does not react. These two findings are viewed as an indication that the nuclei of some of the lymphendotheliocytes may become broken into lobes and assume a polynucleated form. A great many smears both of whole blood and of the leukocytic layers have been treated with benzidin (Method B). An examination of these shows that benzidin reacting mononuclear cells are not present in the blood of the normal rabbit.

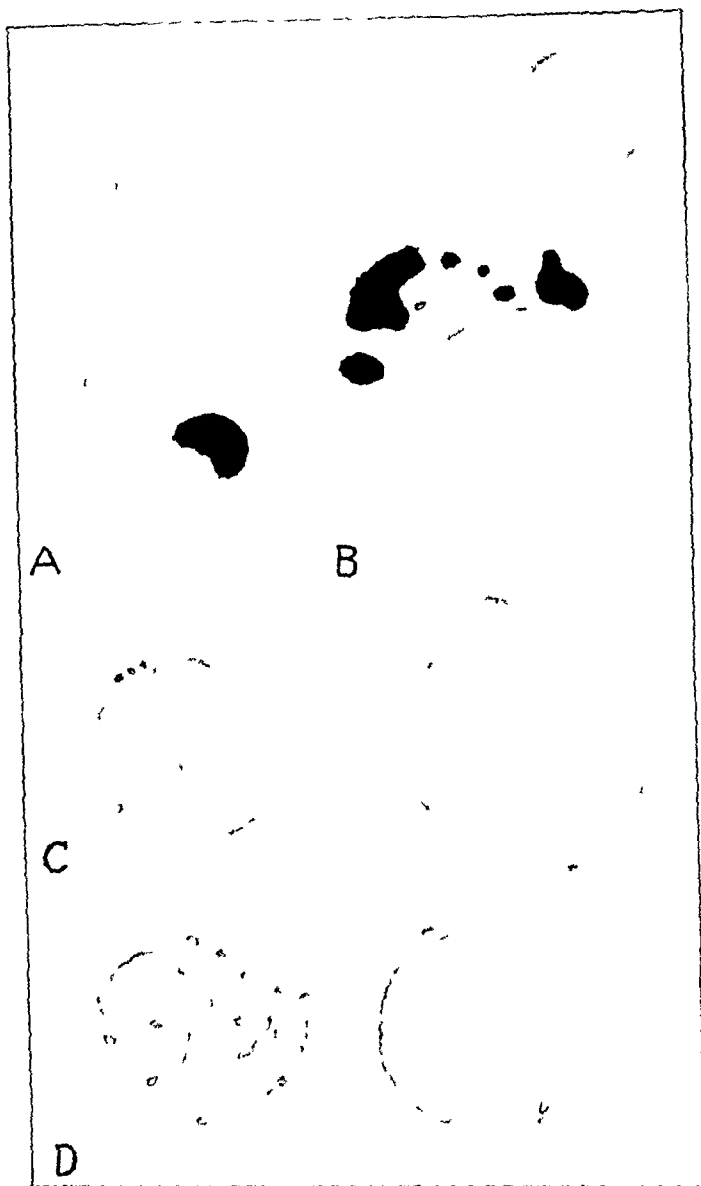


Fig 1—*A* and *B*, smear of peripheral blood of Rabbit H 16 (table) made April 29, 1925, *A*, hemendotheliocyte with one large ink particle in homogenous (hyaline) cytoplasm, red blood corpuscles in same field, *B*, hemendotheliocyte of larger size containing carbon, with a lymphocyte in same field, *C* and *D*, smears of blood of normal rabbit supravitaly stained (Method F), these are both considered to be lymphendotheliocytes since benzidin-reacting cells are not present in the blood of the rabbit, the dye masses are heavy when compared with the dye granules in the lymphocyte and in the neutrophil, Wright's stain, camera lucida. The size of the cells varies in different preparations and in different fields in the same smear, therefore, adjoining cells have been drawn for comparison.

The enlarged lymph nodes from the groin of Rabbit H 16 were examined by the supravital Method G. A great majority of the lymph-endothelial cells (attached) contain no carbon. They are large and present the usual dye focus (Fig 3, A), which often in the fresh preparation appears as a distinct wreath. In an occasional large cell there is a wreath appearance in the paraffin section. In the areas to which the dye has penetrated in a dilute form there is no nuclear staining and the lymphocytes show no dye whatever although the foci in the endothelial cells are very distinct.

MONONUCLEAR PHAGOCYTES OF GUINEA-PIG'S BLOOD

In the blood removed from the right ventricle (thorax opened) of a normal guinea-pig, H 14, there were 4.3 per cent neutral red nonlymphocytic mononuclear cells when examined fresh by Method D. Typical rosettes were not present and there was a greater tendency than in the rabbit for dye granules to appear at several points in the cytoplasm. In the normal guinea-pig and in human blood the dye granules have not been seen to arrange themselves in the rosette or wreath form of Sabin, Doan and Cunningham, although it is usual to find an aggregation of them at one point in the cytoplasm. By Method F in preparations fixed in iodine vapor the relative number of leukocytes is: lymphocytes, 77.3 per cent, polymorphonuclears, 20.3 per cent, and nonlymphocytic neutral red mononuclears, 2.4 per cent. In this instance the discrepancy between the results of examination of fresh and fixed preparations is due to a failure to enumerate many of the lymphocytes in the fresh film. The cells with Kurloff bodies in both the fresh and the fixed preparations are characteristic.

The mononuclear phagocytes of the blood of the normal rabbit do not react with benzidin (Method B). In the peripheral blood of the guinea-pig occasional mononuclear cells that do react to benzidin were previously found by myself and Charlton.¹⁷ The smears should be treated immediately with the benzidin and the diluted solution allowed to react for from five to ten minutes. In the leukocytic layer of a normal guinea-pig, H 14, 0.57 per cent of the leukocytes were found to be benzidin reacting mononuclear cells. Those found were larger in size than the polymorphonuclear leukocytes. Since there were no large mononuclear cells that failed to react to neutral red those positive to the benzidin probably reacted also to the neutral red. In the normal guinea-pig therefore almost all of the nonlymphocytic mononuclear cells are lymphendotheliocytes. Under certain pathologic conditions the relative number

¹⁷ McJunkin, F. A., and Charlton, A. G. An Experimental Endothelial Leukocytosis in Guinea-Pigs, *Arch Int Med* **24**: 295 (Sept) 1919.



Fig 2—Human blood, from H 22, *A* and *B*, from preparation made by peroxidase staining after phagocytosis in vitro (Method A followed by Method B), *A*, lymphendothelocyte and neutrophil, the former devoid of benzidin granules, *B*, benzidin-positive phagocyte, the monocyte of normal human blood, with neutrophil above, *C* and *D*, from a preparation made by supravital staining and subsequent fixation in iodine vapor (Method F), *C*, a lymphocyte with a few fine neutral red granules and a neutral red mononuclear phagocyte, probably a lymphendothelocyte, with heavy dye masses. Such cells in fresh supravital films have no definite rosette but the dye is massed at one place in the cytoplasm. *D*, a polymorphonuclear with small dye granules and a mononuclear, probably a monocyte, with heavy dye granules. Since both lymphendotheliocytes and monocytes react to neutral red, the identification of the two in preparations treated with neutral red alone is not absolute.

of benzidin reacting mononuclears may be much increased. In an earlier report McJunkin and Charlton¹⁷ found as many as 10 per cent in the blood of guinea-pigs in the terminal stages of a general tuberculosis.

In a fresh preparation from a tuberculous guinea-pig, H 13, 8.3 per cent of nonlymphocytic neutral red cells were found in the leukocytic layer prepared from the blood of the right ventricle (Method D). Many of these cells were larger than the polymorphonuclears and often there was a distinct rosette of dye granules made conspicuous by the abundant cytoplasm about the focus of dye. In other large cells the dye granules were much less localized. In direct smears of the whole blood made by snipping the ear there were 0.9 per cent benzidin reacting mononuclear cells. Necropsy of this animal, infected with a human type tubercle bacillus (H 37) of rather low virulence, showed an extensive caseous tuberculosis of the groin lymph nodes with a few small tubercles in the spleen and liver. It has been a common experience in the examination of a number of guinea-pigs moderately tuberculous to find the lymphendotheliocytes considerably increased but with only a slight increase in the benzidin reacting mononuclears. It is only in tuberculous guinea-pigs a few days before death that a distinct increase in the benzidin type of cell takes place. For the demonstration of these cells in the guinea-pig it is much better to make the smears directly from the ear and to treat them at once with benzidin.

In the large mononuclear cells of the epithelioid type present in the tubercles of the spleen and in the tuberculous lymph nodes (Guinea-pig H 13), the neutral red granules viewed in paraffin sections with the supravital dye mordanted in them (Method G) are found to be arranged in beautiful rosette forms (Fig 3, B). The dye usually penetrates only the peripheral portions of the epithelioid tubercle, where all the typical large epithelioid cells appear to be of the rosette type. In the spleen of this animal there is little caseation and the reaction consists almost entirely of these cells. In the liver the tubercles that are smaller are penetrated by cells that appear to be continuous with the vascular endothelium, and in their cytoplasm may be found a few neutral red granules scattered about much as they are in the Kupffer type of endothelial cell. There is direct evidence that both hemendotheliocytes and lymphendotheliocytes take part in tubercle formation in the guinea-pig, but it appears that it is the latter cell that enlarges to become the usual epithelioid cell in the epithelioid type of tubercle in the spleen and lymph node. Lewis¹⁸ observed in incubated blood the development of epithelioid and giant cells from leukocytes present in the normal peripheral blood, including human. It was not shown which type undergoes these transformations.

¹⁸ Lewis, M. R. The Formation of Macrophages, Epithelioid and Giant Cells from Leukocytes of Incubated Blood, *Am J Path* 1:91 (Jan) 1925.

MONONUCLEAR PHAGOCYTES OF HUMAN BLOOD

It is only in human blood that the benzidin reacting leukocyte is the predominating mononuclear phagocyte. Chailton and I¹² compared the peripheral blood by the phagocytic method (Method A) with the same blood treated with benzidin (Method B) and found that the mononuclear cells with ingested carbon and those reacting to benzidin appeared to correspond in number and in general morphology. At this time the

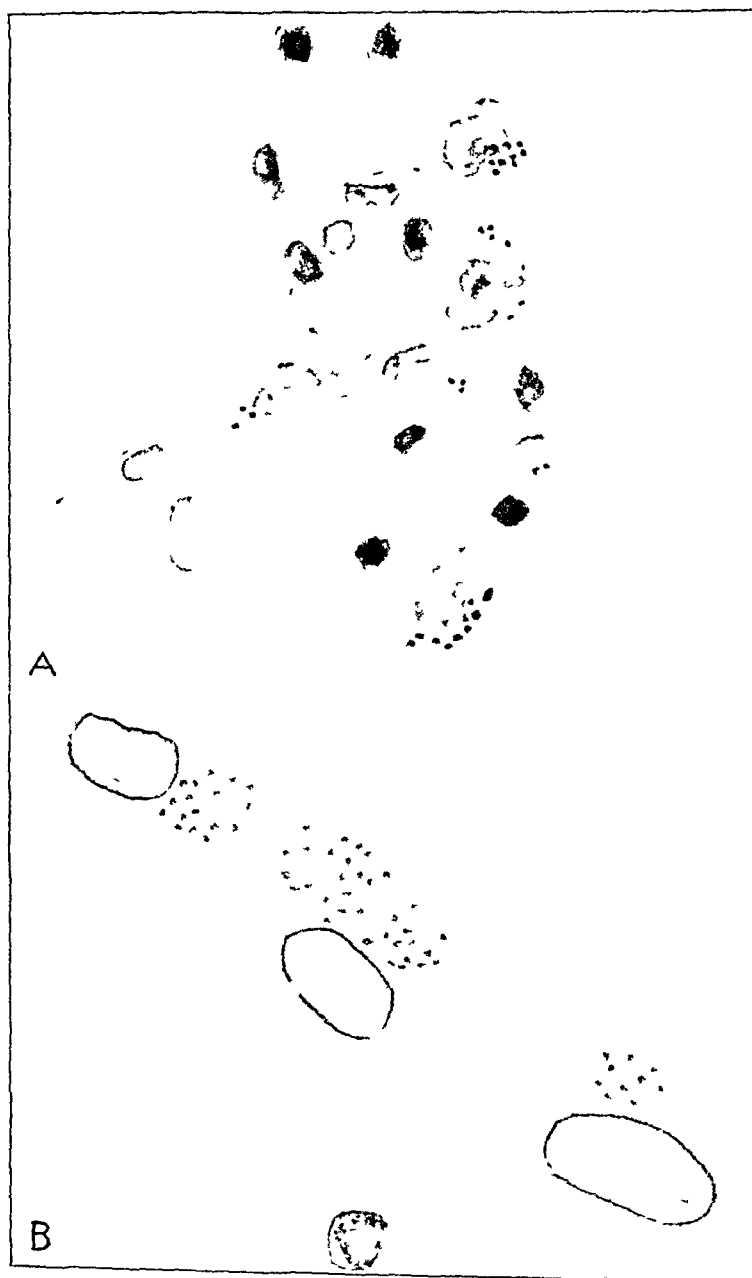


Fig 3—Sections of tissue with supravital dye mordanted in them (Method G) *A*, lymph node removed from groin of Rabbit H 16, the lymphocytes contain no dye but in the cytoplasm of the sinus endothelium there are foci of dye granules, *B*, spleen of tuberculous guinea-pig, H 13, cells at periphery of tubercle made up of epithelioid cells, spherical areas of fine neutral red granules in these large cells highly characteristic, lymphocyte below

conclusion was reached that this benzidin reacting cell was identical with the carbon marked phagocyte that separates from the vascular endothelium. Subsequently, I¹³ found in paraffin sections that neither the lymphatic endothelium nor the vascular endothelium reacted to benzidin. Later when I⁹ determined that the neutral red reacting cells of peritoneal exudates of the rabbit did not color with benzidin it was thought not unlikely that the benzidin reacting cell was a different type from the endothelial leukocytes.

Examination of smears of human lymph nodes removed one hour after death by Method B showed only an occasional benzidin reacting mononuclear cell. In human lymph nodes removed at surgical operation and fixed immediately for examination by Method C, the reticulo-endothelium did not react to benzidin. The occasional mononuclear cell found in the heavy lymph node smears (about 1 per oil immersion field) which reacts to benzidin corresponds morphologically to the benzidin-positive ones of the blood and presents prominent brown granules scattered through the cytoplasm. The other numerous phagocytic cells present do not color with the benzidin. In an examination of lymph glands from a great many individuals by Method C and by staining with hematoxylin only, it has been observed that the endothelial cells lining the medullary sinuses frequently contain small ovoid or spherical bodies, which are apt to appear greenish or brownish. These bodies are sometimes surrounded by a delicate halo that suggests ingested material. In some instances they stain like bacteria while in other nodes they resemble pigment, but a positive reaction for iron has not been obtained. The nature of these structures which have the position in the cells of centrospheres is uncertain.

HUMAN BLOOD IN H 22

Many examinations were made on the normal adult male, H 22. The blood was usually obtained by gently "milking" the finger after deep puncture with a lance. A long series of examinations were first made by subjecting the carbon laden leukocytic layer to vital staining (Method A followed by D) in order to determine the occurrence in the normal peripheral blood of hemendotheliocytes. These examinations ended in the conclusion that this phagocyte with hyaline cytoplasm, which has little affinity for neutral red, is not present in normal human blood. It was stated in a preceding paragraph that the hemendotheliocyte is probably absent from the blood of the rabbit, but in the rabbit the evidence is less conclusive, owing to the partial failure of phagocytosis by Method A.

There is very active phagocytosis of carbon (Method A) by the cells of human blood. In a careful examination of smears of the carbon exposed leukocytes by the peroxidase method with benzidin (Method

B), it soon became evident that mononuclear cells phagocytic for carbon which did not react with the benzidin were present although a majority of the ink marked mononuclear cells did color with the benzidin. In an enumeration of 1,000 cells the following percentages were obtained: lymphocytes (no benzidin), 24.3; neutrophils and eosinophils (heavy benzidin with no carbon in eosinophils and little carbon in neutrophils), 70.8; mononuclear phagocytes (heavy benzidin and much carbon), 3.4; mononuclear phagocytes (no benzidin and much carbon), 1.1; and polymorphonuclear cells with no benzidin (unclassified), 0.4. Taken as groups the benzidin mononuclear phagocytes appear to differ somewhat from the nonbenzidin ones. The benzidin-positive cells average somewhat larger in size and their nuclear contour is often broken by deep indentations (Fig. 2, B), while the nucleus of the benzidin-negative cell may present only a flattened surface toward a wider expanse of cytoplasm, or the nuclear outline may be broken by one or more shallow V shaped depressions (Fig. 2, A). In the incubated specimen, in which ameboid motion is likely to distort both nuclear and cytoplasmic outlines, there appears to be no feature other than the reaction to benzidin which will serve to distinguish the two types of cells. It also is certain that some of the nonbenzidin phagocytes can be differentiated from lymphocytes only by the ingested carbon. Where the carbon particles are very numerous in the nonbenzidin cells a faint brownish tinge in the region of masses of small carbon particles may sometimes be seen. This is seen also in smears not treated with benzidin as well as in the phagocytes of rabbit's blood, in which no benzidin reaction is present. This slight brownish discoloration is due to the finest ink particles and is readily distinguished from the coarser particles of benzidin reacting cytoplasm.

A second method has been employed to determine by direct microscopic examination the occurrence in the blood of neutral red reacting phagocytes that do not color with benzidin. Smears of the leukocytic layer (not treated with ink) supravitaly stained (Method D) when dry were examined with oil immersion lens. The dye could be seen but was less distinct than in the fresh preparation. A cell or a small group of cells was placed at the center of a field under the oil immersion objective and then with the aid of low power lens a ring about the cell was scratched in the film with a teasing needle. The location of the cells "spotted" was noted and the preparation treated with benzidin (Method B) followed by hematoxylin. The red was dissolved by the alcoholic benzidin, but the heavier neutral red reacting masses in cells positive to benzidin colored a greenish tinge instead of the usual brown. The red was completely removed from the cytoplasm of cells that did not react to the benzidin. In five smears from one leukocytic layer sixteen mono-

nuclear cells containing heavy masses of neutral red (Fig 2, C and D) were located and afterwards treated with benzidin. Four of the sixteen were devoid of brown coloring while the other twelve showed a heavy greenish staining with the benzidin. The statements of the general morphology of the two groups of phagocytes identified by the phagocytic and peroxydase methods apply to these two groups. The nuclei of the benzidin cells are more broken in outline and are covered with oxydase granules. They stain much like the nuclei of the polymorphonuclear neutrophils.

COMMENT AND SUMMARY

A procedure of first importance in obtaining information in regard to cells present in the blood in scant numbers is to concentrate them in a leukocytic layer with the least possible injury to the cells. The appearance in the peripheral blood of the hyaline type of leukocyte derived from the endothelium of the blood capillaries, the hemendotheliocyte, appears to be pathologic and this cell has been seen only in rabbits given huge doses of carbon suspension. The cell is identical with the hyaline type observed by me⁹ in the peritoneal exudates. Since it is marked by the ink and contains little or no neutral red, this cell presents a characteristic appearance in supravital films in both blood and peritoneal exudates. The blood capillary type of endothelial cell, such as the Kupffer cell, is in these animals heavily marked with ink and reacts little to neutral red. The phagocytes anchored in the splenic sinuses, which have ingested enormous quantities of carbon, react little to the dye. The results obtained by vital staining with colloidal dyes, by marking the vascular endothelium with carbon in suspension and by supravital staining support the view that this hyaline type of phagocyte arises from the endothelium of the capillary blood vessels and sinuses. The only specific designation for such a cell appearing in the peripheral blood is that of the hemal type of endothelial leukocyte or the hemendotheliocyte.

The neutral red reacting cell, on the basis of information previously presented,⁹ is considered to be a derivative of lymphatic reticulo-endothelium and may therefore be spoken of specifically as the endothelial leukocyte of lymphatic origin, or as the lymphendotheliocyte. The number of lymphendotheliocytes in the blood has been increased experimentally in two ways: first, in the rabbit by injection of whole rabbit's blood into the peritoneal cavity (maximum of 7 per cent in case of Rabbit H 16), and, second, in the guinea-pig by infecting with *Bacillus tuberculosis* (maximum of 8.3 per cent in a tuberculous guinea-pig, H 13). The association of these experimental lymphendotheliocyte increases with definite lymph node enlargement is significant. The groin

lymph nodes of Rabbit H 16 (table) measured 3 by 5 by 9 mm and microscopically the reticulo-endothelium was much hypertrophied and rosettes of neutral red granules were present in the cytoplasm of some of the lining cells (Fig 3, *A*) In the tubercles of guinea-pigs a beautiful rosette grouping of the granules was seen in the large epithelioid cells (Fig 3, *B*) Only the inguinal, iliac, cervical and tracheobronchial lymph nodes have been examined but it seems likely that wherever anastomosing lymph channels of a capillary type occur these phagocytes may arise In the secondary lymph nodules of lymph nodes the neutral red reaction has been obtained only in the reticular cells at the periphery, but usually the dye has not penetrated in a satisfactory manner to the germinal centers The lymphocytes in supravital films of blood often contain from one to several neutral red granules in a small focus of cytoplasm, but in the cellular "juice" expressed from lymph nodes on dry dye films the excess of dye is absorbed by the numerous cells and only the larger reticular cells show a focus of dye granules Likewise, when the dye is injected into the lymph nodes for the preparation of paraffin sections, only the reticulo-endothelium contains the dye Since the lymphoid tissue of the spleen is usually stated to contain reticulum this organ has been given special attention Cunningham, Sabin and Doan⁷ found from 15 to 20 per cent of the cells obtained from the spleen under anesthesia by puncture with a fine capillary pipet to be of the rosette type My experience⁹ was that these cells were present in about the same proportion to the other cells that they were in the blood In supravital stained paraffin sections typical rosette cells have not been demonstrated in the splenic corpuscles Splenectomy does not influence the number of the rosette type of cell appearing in peritoneal exudates The phagocytic activity of the lymph-endotheliocytes appears to serve as a certain means of separating them from the lymphocytes, but it is not certain that both may not arise from a younger common stem cell, although no evidence has been obtained to support such origin

The third type of mononuclear phagocyte appearing in peripheral blood reacts both to neutral red and to benzidin Naegeli¹⁰ named these cells monocytes and noted that they give the oxydase reaction He did not, however, recognize any type of mononuclear phagocyte other than the monocyte Since others, among whom are Schilling,¹¹ have found that cells of this general type did not give the oxydase reaction, it may again be pointed out that it is necessary to specify the particular form of this reaction employed However, it is possible that

19 Schilling, V Ueber hochgradige Monozyten mit makrophagen bei Endocarditis und uber die Herkunft der grossen Mononuclearen, *Ztschr f klin Med* 88 377, 1919

Schilling, for example in his cases of infectious endocarditis, was dealing with lymphendotheliocytes or with hemendotheliocytes, both of which fail to react with benzidin. Human bone marrow obtained at necropsy has been examined by Method C. It has usually been expressed from the cut ends of the ribs by crushing with bone forceps and at times has been placed in the fixing fluid within one hour after death. In sections of tissue the reaction of the benzidin is fully as intense as in smears but the brown is diffuse rather than in the form of individual granules. The presence of numerous myeloblastic cells makes difficult the determination of a second type of benzidin reacting cell in marrow. There are always present a considerable number of cells reacting less intensely than the usual myelocyte and the nuclei of many of these have the irregularity of outline seen in the benzidin-positive mononuclear cells of the blood. In the splenic pulp I¹³ previously observed the presence of cells that resembled the blood mononuclears more than they did the myelocytes.

That the spleen is not the sole source of these cells is shown by the examination of the blood of an adult, E. M., from the surgical service of Barnes Hospital, St. Louis, before and after splenectomy. Nov. 4, 1924, before splenectomy, there were 19 per cent of benzidin-positive mononuclear cells, three days after splenectomy, 27 per cent, eight days after splenectomy, 23 per cent. June 22, 1925, there were 43 per cent of mononuclear cells reacting to benzidin. In all except the last examination there was a relative increase in polymorphonuclears and the white cells per cubic millimeter were 21,000, 27,000 and 22,000, respectively, on the three days on which the first three examinations were made. The clinical diagnosis was purpura.

Since these cells are demonstrable only in the bone marrow, spleen and blood stream it seems likely that they arise chiefly in the bone marrow and to a less extent in the splenic pulp. Their property of ingesting much carbon in citrated blood in which there is very little phagocytosis by the polymorphonuclears may be interpreted to mean that these cells are not of the usual myeloblastic series. The alternative explanation is that there is a stage in the differentiation of the myelocyte when the cell has an unusual affinity for carbon. A reaction to benzidin less intense than that of the neutrophilic myelocytes also suggests that they are a distinctive type of cell. It may prove that a close relationship exists between these and the myeloblastic series, and Evans²⁰ found neutrophilic leukocytoses and increases in these cells to be associated in some instances. The term monocyte proposed by

²⁰ Evans, F. A. Observations on the Origin and Status of the So-Called Transitional White Blood Cells, *Arch. Int. Med.* **18**: 692 (Nov.) 1916.

Naegeli for this peroxidase-reacting mononuclear leukocyte should be applied to this particular cell alone and not to the nonbenzidin reacting phagocytes arising from endothelium

CONCLUSIONS

1 Monocytes, mononuclear benzidin-positive phagocytes reacting to neutral red by the method of supravital staining, are present in normal human blood and an occasional one is present in the blood of the normal guinea-pig. These cells have not been demonstrated in rabbit's blood. They are increased in the blood of the guinea-pig during the terminal stage of tuberculosis. Since they are found only in the bone-marrow and spleen these fixed tissues are regarded as the probable source for their production.

2 Lymphendotheliocytes, benzidin-negative mononuclear phagocytes that by the method of supravital staining react to neutral red (sometimes with arrangement of the dye granules in the form of a rosette), are present in the peripheral blood of the normal guinea-pig and rabbit and in human blood. They are increased in the blood of the rabbit by intraperitoneal injections of whole rabbit's blood and in the guinea-pig's blood during tuberculous infection. They arise from the lymphatic reticulo-endothelium. These leukocytes become transformed into the epithelioid cells of tubercles with preservation of their characteristic reaction to neutral red.

3 Hemendotheliocytes, benzidin-negative mononuclear phagocytes not reacting by the method of supravital staining to neutral red or reacting slightly to neutral red with the appearance of irregular dye cytoplasmic granules, have not been demonstrated in the normal peripheral blood. They appear in the blood of the rabbit following intravenous injections of India ink. These phagocytes, which react earlier and more intensely to intravenous carbon in suspension and to colloidal dyes than do other cells, probably arise from the blood vascular endothelium.

4 By direct examination, therefore, three kinds of mononuclear phagocytes may be demonstrated in the circulating blood, two of which are found under normal conditions. Less direct evidence points to the conclusions that the benzidin-reacting phagocytes are derived from the bone marrow and the spleen, and that the two benzidin-negative phagocytes arise from the lymph nodes and from the endothelium of capillary blood vessels, respectively.

THE ETIOLOGY OF CHRONIC ULCERATIVE COLITIS

EXPERIMENTAL STUDIES WITH SUGGESTIONS FOR A MORE
RATIONAL FORM OF TREATMENT *

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AND

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A year ago we believed we had isolated the organism responsible for at least a prominent part in the cause of chronic ulcerative colitis of the so-called idiopathic type ¹

The organism was obtained with sterile swabs through the proctoscope from the depths of an ulcer of the colon, after the latter had been thoroughly cleansed by repeated washings and the top of the ulcer had been carefully wiped with a preliminary swab. Cultures of stools failed to yield the organism but in cultures of the rectal discharge of mucus, pus and blood it was often found as the vastly predominating bacterium. The material obtained was cultured immediately in warm dextrose brain broth,² blood agar and lactose agar and incubated for twenty-four hours. All mediums were titrated to a p_H 7.4. The organism isolated in this way as the predominating bacterium and often in pure cultures is a gram-positive, lancet shaped diplococcus, growing in twos and fours (Fig 1) and has been observed with a capsule occasionally, thus morphologically resembling a pneumococcus. It is not bile soluble. Twenty strains examined have failed to ferment inulin or mannite but have always fermented dextrose, lactose, saccharose, maltose, raffinose, salicin and acidified milk, shortly after their isolation. When retested weeks or months after isolation, the fermentation of the last six sugars has varied. The diagnostic pneumococcus serums of Types I, II and III have failed to agglutinate a number of typical strains. On blood agar the diplococcus grows as an alpha-hemolytic streptococcus (Fig 2). Agglutinins and precipitins have been demonstrated in the blood of rabbits immunized with various strains of this organism. Cross agglutination and precipitation have been found among at least six strains examined.

¹ From the division of medicine, Mayo Clinic

* Read before the Section on Pharmacology and Therapeutics at the Seventy-Sixth Annual Session of the American Medical Association, Atlantic City, N J, May, 1925

¹ Bargaen, J A. Experimental Studies on the Etiology of Chronic Ulcerative Colitis, J A M A 83 332-336 (Aug 2) 1924

² Rosenow, E C. Studies on Elective Localization, Focal Infection with Special Reference to Oral Sepsis, J Dent Res 1 205-268, 1919

In two years, cultures have been made from the lesions of sixty-eight patients with chronic ulcerative colitis. In 80 per cent of the cases, such a diplococcus has been isolated.

Cultures also have been made by swabbing the healthy colon mucosa of twenty persons presenting themselves for proctoscopic examination because of hemorrhoids or other rectal complaints, and only once were diplococci of similar characteristics isolated.

Smears of the material obtained from the ulcers consist of pus cells and red blood cells but, in the majority of them, diplococci of similar structure can be recognized in appreciable numbers.



Fig 1—Freshly isolated culture of the diplococcus described

Since our last report 139 rabbits have been injected intravenously with dextrose brain broth cultures in amounts varying from 5 to 15 c c. Lesions developed in the colon in forty-five of these without lesions being grossly demonstrable elsewhere. Fully as many developed diarrhea, these rabbits were injected with pure cultures of rapidly isolated diplococci. Rosenow and his co-workers, using a similar technic, have injected large numbers of rabbits with various strains of streptococci and have never found lesions in the colon such as these.

The organism sometimes localizes in the gallbladder of rabbits. Eighteen rabbits were fed on vitamin-free food (crushed oats and water or autoclaved rice and water, according to the methods of McCairi-

son³) for two weeks. At the end of this time, nine were injected intravenously with 5 c c of a dextrose brain broth culture of the freshly isolated diplococcus. All were dead within five days, eight had lesions of the colon, from submucous hemorrhages to minute ulcers. Three of the eight had empyema of the gallbladder from which a pure culture of the diplococcus was isolated.



Fig 3—Three rabbits fed on vitamin-free food for two weeks and three days

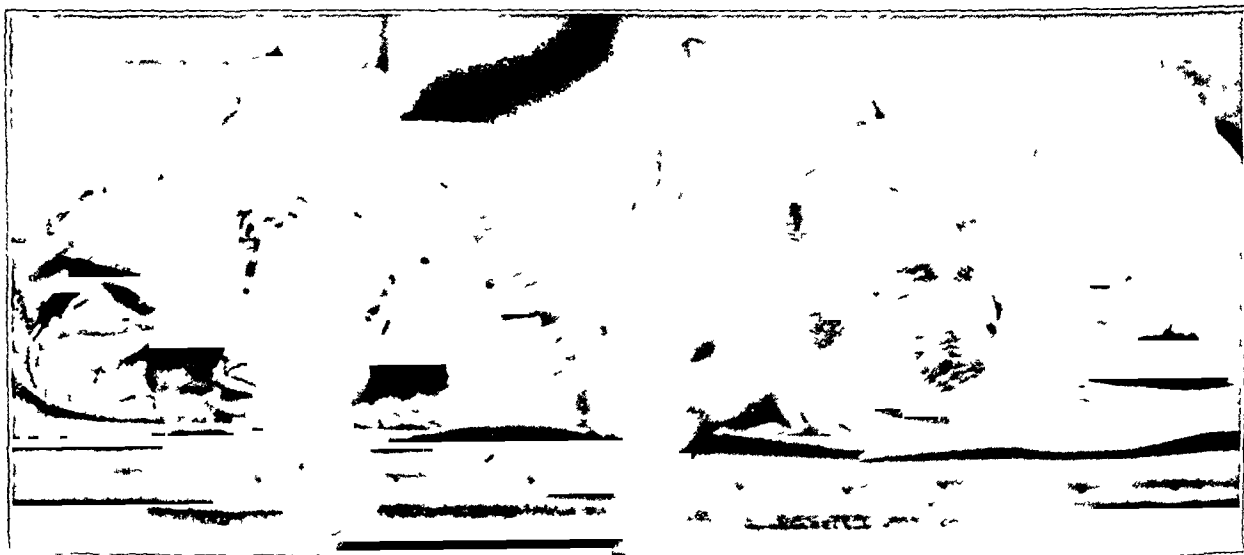


Fig 4—Three rabbits three days after injection with the diplococcus fed on vitamin-free food for three weeks

Of the other nine, five died, apparently from starvation with certain of the symptoms of a deficiency syndrome, while four lived two months on the diet (Figs 3 and 4).

³ McCarrison, Robert. *Studies in Deficiency Disease*, London, Frowde, Hodder and Stoughton, 1921.



Fig 2—Typical twenty-four hour colonies isolated from acute lesions of the colon in pure culture



The culture of the diplococcus isolated from these gallbladders was injected intravenously into two young dogs, weighing 4 and 6 kg, respectively. Repeated daily injections were given in 12, 20, 20, 20, 20, and 30 c c in an effort to produce a chronic disease more like that in human beings, since the lesions in rabbits were rather of the acute fulminating type. On the fourth day the dogs began passing bloody mucus, on the sixth there was marked rectal prolapse exposing an edematous, granular, easily bleeding, superficially ulcerated mucosa, the typical picture of early ulcerative colitis. At the end of two weeks, one of the dogs began to improve, and repeated large doses of the organism had no effect. When it was killed at the end of three weeks, a healing ulcerative colitis of the lower 6 inches (15.2 cm) of the colon was found. In the other dog colon changes were demonstrable by roentgen ray with the barium enema at the end of three weeks, after which he too began to get well (Fig 5).

The possibility that gallbladders might act as a focus for harboring these diplococci made it seem reasonable that other distant foci in human beings might be of importance.

A woman, aged 26, entered the Mayo Clinic, Dec 30, 1924, with a history of having had bloody diarrhea for three years. Eight months previous to her first spell of diarrhea, she had had a tooth devitalized and it had given her some trouble ever since. In the autumn of 1921, she had diarrhea for two weeks, without apparent cause. In the spring of 1922, she had diarrhea for six weeks. In the spring of 1923, her diarrhea recurred and lasted until her admission to the clinic. From twelve to twenty stools a day were often passed, with mucus, blood and pus. Chronic ulcerative colitis was discovered, a diplococcus was isolated from the rectal lesions, and a vaccine prepared for her. When 0.1 c c of it was given subcutaneously there was a marked increase in the diarrhea. Several days later 0.2 c c was given with an even more severe diarrhea and marked soreness of her dead tooth in which a periapical abscess was revealed by the roentgen ray. The vaccine was then discontinued and her septic tonsils and, later, her abscessed tooth were removed. The extraction was followed by a severe upset of the bowel. In the culture of the periapical abscess, diplococci of the variety described predominated in large numbers, with short chain streptococci making up the remainder of the culture.

The six hour glucose brain broth subculture of the twelve hour culture of the pus from this periapical abscess was injected intravenously into a rabbit, and in twenty-four hours the animal was dead. Extensive submucous hemorrhages and minute ulcers of the lower three-fourths of the colon were found (Fig 6). A pure culture of the diplococcus isolated from the mesenteric lymph nodes, which were very

large and acutely hemorrhagic, was injected into two young dogs, weighing 72 and 11 kg, respectively. Both were dead in less than twenty-four hours, the floor of the cage being covered with blood. Massive submucous hemorrhages were found in the colon and disseminated hemorrhages in the lower ileum and in the upper jejunum.

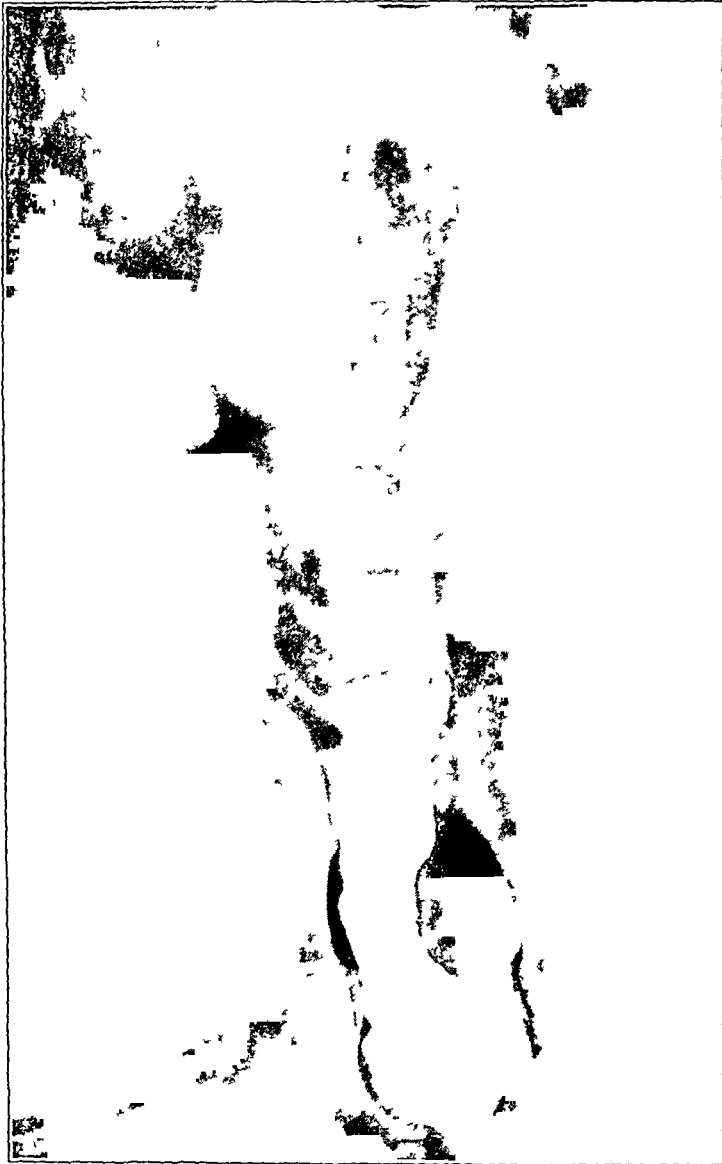


Fig 5—Dog's colon showing ulcerative colitis by barium enema three weeks after initial injection of diplococci

(Fig 7) In neither the rabbit nor the dogs were lesions found elsewhere

Since this striking result we have seen repeated severe bowel reactions with acute bloody dysentery, following extraction of such foci as teeth and tonsils. One young woman after a series of injections of

vaccine filtrate was doing well, with one or two stools daily and no blood, when her septic tonsils were removed. Several days later, following the administration of 0.6 c.c. of vaccine, she had a terrific reaction with fifteen bloody stools in twenty-four hours and marked prostration. After she had rested a week in bed and her bowels had gradually quieted, 0.3 c.c. of vaccine was administered, the dose being increased by 0.1 c.c. every third day. In two weeks she felt entirely well, and had one formed movement a day. She has remained well.

These facts clearly demonstrate the importance of distant foci in the etiology of this disease. How important they may be in its continu-

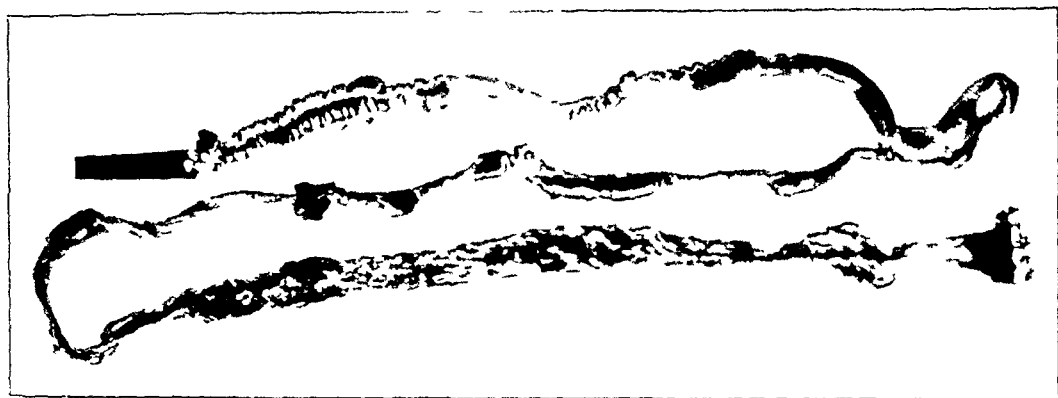


Fig 6—Extensive submucous hemorrhages and minute ulcers of lower three-fourths of colon of rabbit injected intravenously with organism obtained from periapical abscess



Fig 7—Massive submucous hemorrhage of dog's colon injected with pure culture from mesenteric glands of the same rabbit

ance, it is difficult to say, but their part in the early stages certainly seems established.

Lesions observed in their early stages have appeared as milium abscesses (Fig 7). A few days later superficial ulceration of the tops of the abscesses and their confluence produce the typical granular appearance. From such lesions, seen on the protruding bowel after ileostomy, a pure culture of the diplococcus has been isolated in the minute drop of pus obtained from the tiny abscesses in the mucosa. These repeated "showers" of infection and healing give one a clear

conception of how the thick layer of granulation tissue and underlying fibrous tissue may be formed, as it is seen in cross sections of the colons of these patients after death. In sections through the lesions stained for bacteria, large numbers of gram-positive diplococci of a structure similar to those described are commonly seen (Fig 8)

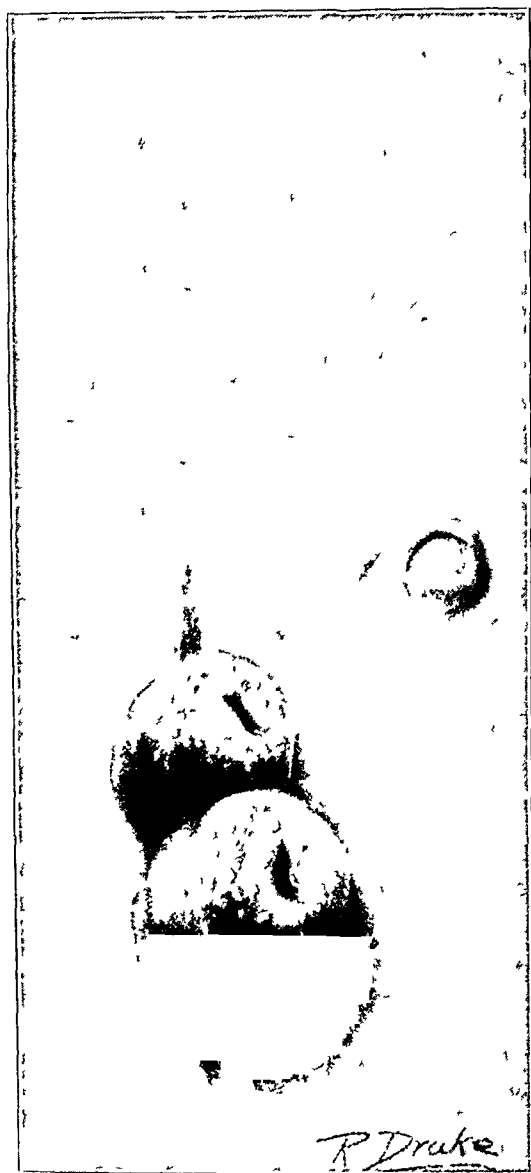


Fig 8—Five and six day old lesions of protruding portion of intestine after ileostomy in case of chronic ulcerative colitis

With these facts before us, it would seem logical that some form of immunization against this diplococcus offers hope for patients suffering from chronic ulcerative colitis. This has been attempted by preparing vaccines and vaccine filtrates according to the methods of Rosenow. Vaccines are prepared by heavily inoculating 8 ounce (236 c c) bottles

of 1 per cent dextrose broth, incubating for twenty-four hours, centrifugating and making a thick glycerin suspension of the bacterial precipitate. To 24 cc of sterile physiologic sodium chloride solution is added 0.2 cc of the glycerin suspension and 0.08 cc of triceosote, making a suspension of 2,000,000,000 dead bacteria for each cubic centimeter. This is allowed to stand in the icebox over night. Vaccine filtrates are prepared by passing the original dextrose broth culture through a Berkefeld filter and adding 0.04 cc of triceosote to the sterile filtrate.

Of either material, 0.1 cc is given as the initial dose. Patients who had slight systemic reaction with the small doses have obtained the best results. Recently we have used more filtrates, seemingly with better results. Injections are made subcutaneously every third day, beginning with 0.1 cc, the dose being increased by 0.1 cc at each injection up to 1 or 1.5 cc according to the patient's reaction to the toxin. In two or three weeks, the diarrhea subsides and the patients improve, often very rapidly. Twelve patients have been dismissed clinically well and, on proctoscopic examination, at least much better; nine have improved, others have either not taken the vaccine or filtrate, or not enough of it to justify a statement with regard to results (Tables 1 and 2).

In conjunction with the vaccine treatment, we have, of course, continued our usual treatment of the disease. When the lesions are low enough to be reached by proctoscope, local treatment with 1:4,000 acriflavin, 1 per cent mercurochrome, silver nitrate, lunar caustic and similar medicaments have been applied. Irrigations with hot water, warm physiologic solutions of sodium chloride, aigylol, acriflavin 1:4,000, and instillations of witch-hazel and bismuth in olive oil also have been used. An important adjunct to the treatment is the administration of tincture of iodine by mouth, according to the methods of one of us,⁴ and kaolin in doses of from 1 to 1.5 ounces. The nature of the action of the former is not definitely known, but a leukocytosis is often produced by its use. The kaolin presumably acts as an adsorbent and may have an antiperistaltic action. We have allowed these patients a rather generous diet, avoiding only such food as they themselves have found to upset their bowels.

SUMMARY

A gram-positive diplococcus has been isolated in 80 per cent of cases of chronic ulcerative colitis. This organism has been obtained in pure culture from the early lesions, in an acute exacerbation of the disease, from the depths of chronic ulcers, and occasionally from distant

⁴ Logan, A. H. Three Cases of Ulcerative Colitis Cured with Iodine, *Med Clin N. A.* 7:105-112 (July) 1923.

TABLE 1—*Patients with Chronic Ulcerative Colitis Treated with Vaccines and Vaccine Filtrates**Patients Clinically Well on Dismissal**

Case	Sex	Age, Years	Objectively Well, Months	Symptoms Pre-vious to Admission	Roentgen-Ray Findings in Colon on Admission	Proctoscopic Examination	
						On Admission	On Dismissal
1	F	31	7	5 months	Chronic ulcerative colitis	Chronic ulcerative colitis	Well
2	F	57	5	12 years	Chronic ulcerative colitis of recto sigmoid	Chronic ulcerative colitis	Improved 30 per cent
3	M	43	13	2 years	Negative	Chronic ulcerative colitis	Improved 50 per cent
4	F	30	8	6 years	Chronic ulcerative colitis of left half of colon	Chronic ulcerative colitis	Improved 75 per cent
5	M	40	4	17 months	Chronic ulcerative colitis of entire colon	Chronic ulcerative colitis	Improved 30 per cent
6	M		9	1 year	Doubtful	Chronic ulcerative colitis	Well
7	M	29	3	7 years	Negative	Chronic ulcerative colitis	Well
8	F	25	2	7 years	Chronic ulcerative colitis of left half of colon	Chronic ulcerative colitis	Improved 30 per cent
9	F	36	1	3 1/2 years	Negative	Chronic ulcerative colitis	Well
10	F	28	2	3 years	Chronic ulcerative colitis of recto sigmoid	Chronic ulcerative colitis	Improved 50 per cent
11	F	16	2	3 months	Negative	Chronic ulcerative colitis	Well
12	F	40	3	6 years	Negative	Chronic ulcerative proctitis	Practically well

* One well formed stool daily

TABLE 2—*Patients with Chronic Ulcerative Colitis Treated with Vaccines and Vaccine Filtrates**Patients Clinically Improved on Dismissal*

Case	Months	Duration of Symptoms	Roentgen Ray Findings in Colon on Admission	Proctoscopic Examination	
				On Admission	On Dismissal
1	3	6 years	Chronic ulcerative colitis of left half of colon	Chronic ulcerative colitis	Slight improvement
2	12	25 months	Ulcerative colitis of sigmoid	Chronic ulcerative colitis	Improved 50 per cent
3	4	11 years	Chronic ulcerative colitis of entire colon	Chronic ulcerative colitis 3+	Improved 50 per cent
4	2	1 year	Chronic ulcerative colitis	Chronic ulcerative colitis 2+	Slight improvement
5	2	20 months	Chronic ulcerative colitis	Anal ulcers	
6	2	16 years	Chronic ulcerative colitis of entire colon	Chronic ulcerative colitis with marked contraction	Improved
7	2	4 years	Chronic ulcerative colitis	Chronic ulcerative colitis 3	Improved
8	3	1 year	Negative	Granular procto sigmoiditis	Improved 25 per cent
9	2	10 years	Negative	Chronic ulcerative proctitis	Improved 50 per cent

foci elsewhere in the body. If searched for at the proper time when the lesions are active, we believe that the organism would always be found. By the intravenous injection of the organism, lesions like those in human beings have been produced in rabbits and dogs, the lesions in the latter approaching the chronic stage of the disease in human beings. The finding of this organism in distant foci and the production of acute lesions in the colon could account for the repeated exacerbations of the disease and the pathologic findings of layer on layer of the granulation and fibrous tissue, replacing the colon mucosa.

From these studies, it would seem that the hope of control of this disease lies in (1) removal of the distant foci of infection, (2) immun-

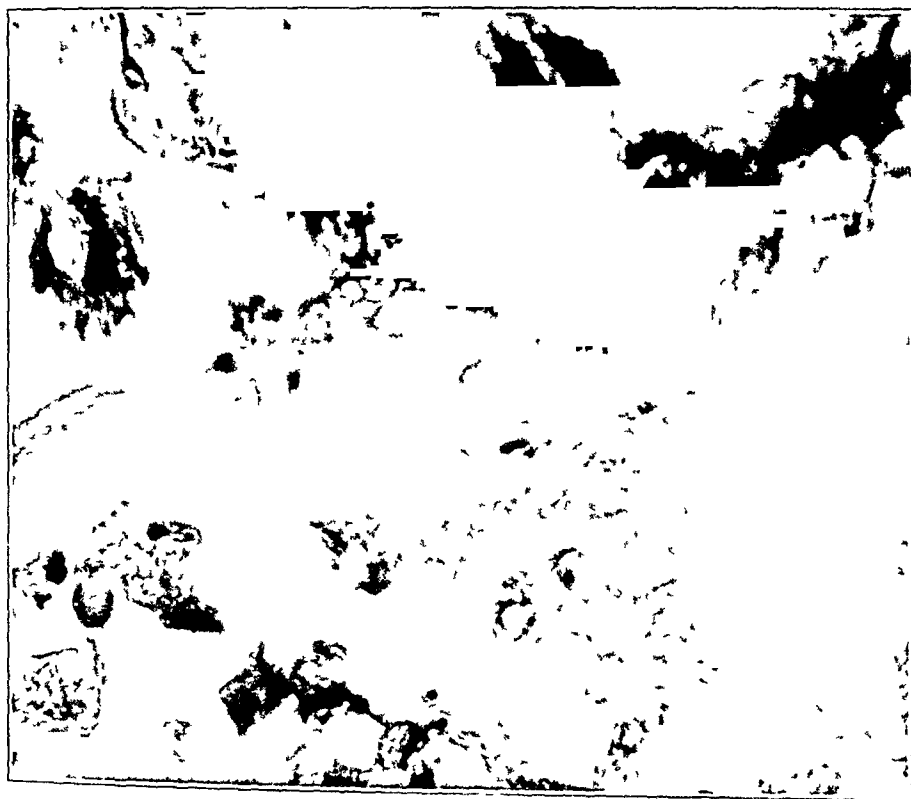


Fig 9—Section through lesion of colon of chronic ulcerative colitis, showing diplococci

ization in some form against the causative organisms, (3) local and topical application and irrigation, (4) the empiric use of drugs, such as tincture of iodine, (5) the administration of some adsorbent like kaolin by mouth, and (6) a nonirritating yet general diet.

REPORT OF CASES

CASE 1—A woman, aged 31, came to the clinic, May 19, 1924, with a history of having had bloody diarrhea with mucus and pus during the last year. She had had from fifteen to forty stools in twenty-four hours with severe abdominal cramps and rectal tenesmus. Except for two weeks of freedom, two months before coming to the clinic, her trouble had steadily progressed with loss of 28 pounds (127 kg) in weight.

A very diffuse, active ulcerative proctosigmoiditis, with activity 3 on a basis of 4, was found by proctoscopic examination. The roentgen ray demonstrated that the disease included the transverse colon. Repeated examinations of the stool failed to reveal parasites or the bacilli of tuberculosis, but gram-positive diplococci were isolated by culture. Because of the patient's acute condition, she was admitted to the hospital and given daily hot water irrigations (at 120 degrees F), argyrol instillations, kaolin and tincture of iodine by mouth and autogenous vaccine. The vaccine treatment was continued for four months. She reported, Jan 7, 1925, for reexamination. By proctoscopic examination the bowel was entirely healed. The roentgenogram of the colon was negative. She had one well formed stool daily and was clinically well.

CASE 2—A woman, aged 37, came to the clinic, Nov 24, 1924, with a history of rectal bleeding which had become progressively worse during the last twelve years, so that during the last six months she had lost 25 pounds (11.3 kg), and was passing six or seven stools with much blood, mucus and pus daily.

The proctoscopic examination revealed diffuse inflammation of the rectum and sigmoid, contraction to one-third of their usual lumen, and irregular ulcers with membranous bases and bleeding margins. The roentgen ray revealed a large dilated colon with a lesion of the sigmoid. A diagnosis of chronic ulcerative colitis was made. Repeated examinations of the stool failed to reveal the bacilli of tuberculosis or amebas, but cultures of the rectal discharge yielded a large predominance of gram-positive diplococci. The patient had slight anemia, the leukocytes numbered 10,100. She was given autogenous vaccine, local topical applications of witch-hazel, 15 per cent silver nitrate solution, lactose three times daily, and tincture of iodine by mouth. Dec 18, 1924, she left the clinic showing 30 per cent improvement on proctoscopic examination. She wrote after two months that she was entirely well, and a recent report was similar in tone.

CASE 3—A man, aged 43, came the clinic in May, 1922, with a history of bloody diarrhea with mucus and pus for two months. He had marked urgency and tenesmus with passage.

Repeated tests of the stool did not reveal parasites or acid-fast bacilli. Roentgenograms revealed disease of the rectosigmoid and median pelvic colon. After proctoscopic examination, chronic ulcerative colitis was diagnosed. The patient was given the usual local treatment and emetin hydrochlorid hypodermically, and improved slightly. He then returned at intervals of several months with acute exacerbations and early in 1924 a gram-positive diplococcus was isolated by culture. A vaccine was prepared and administered. The usual local treatment was continued, namely, instillations of witch-hazel, with irrigations of hot water and applications of lunar caustic. When seen in August, 1924, he was entirely well clinically and the proctoscope showed healed lesions. He has remained well to this time.

CASE 4—A woman, aged 28, came to the clinic because she had been troubled for four years with intermittent bloody diarrhea, up to twenty stools daily, with much mucus, pus and blood. The first spell lasted two weeks, the next six weeks, and the last continued for one and a half years, until the time of admission.

Repeated tests of the stool failed to show the presence of parasites, but from the scrapings from the lesions a predominance of gram-positive diplococci was cultured. Roentgen-ray examination of the colon revealed a lesion of the rectosigmoid suggestive of ulcerative colitis, and after proctoscopic examination a diagnosis of ulcerative colitis was made. The patient also had a chronic polyarthritis of several years' standing. One tooth with a periapical abscess and two impacted molars were discovered, the condition of the tonsils was questionable.

With the pus of the periapical abscess, lesions were produced in rabbits. A marked bowel reaction followed the extraction of the teeth, but soon afterward improvement of the arthritis and colitis was manifested. She was given a course of vaccine, at first with a violent reaction even with 0.1 c.c., but the reactions gradually diminished. She was given local treatments with 15 per cent silver

nitrate solution, lunar caustic and witch-hazel. On dismissal the proctoscope showed healed lesions and she had recovered clinically from her arthritis and colitis.

CASE 5—A man, aged 29, came to the clinic, Feb. 4, 1925, because of persistent passage of blood and pus by rectum since his discharge from the army in 1918. He had difficulty in passing stools, but he had had from three to five passages of blood and pus daily. In two years he had lost only 6 pounds (2.7 kg.) and ate well. His only other symptoms were severe distress from gas and frequent rectal spasms.

On proctoscopic examination a granular, easily bleeding mucous membrane of the colon was found, covered by much exudate. A diagnosis of proctosigmoiditis was made. The patient was placed on an anticonstipation regimen, had daily local applications of 15 per cent silver nitrate solution, alternating with 3 per cent mercurochrome or lunar caustic, with instillations of witch-hazel and a series of injections of vaccine filtrate. March 11, 1925, he was dismissed clinically well and the proctoscope revealed healed lesions. He was advised to continue the vaccine treatment and to have both infected tonsils removed at home.

CASE 6—A man, aged 40, came to the clinic in January, 1924, because of four months of bloody diarrhea of very gradual onset. During the last three months he had been passing ten or more stools a day with blood, pus and mucus, with much tenesmus. He had lost only 9 pounds (4.1 kg.), and except for his abdominal distress had no complaints.

A diagnosis of chronic ulcerative colitis was made after proctoscopic and roentgenographic examinations with the barium enemas. The entire colon was found to be affected. Periapical abscesses were found around two teeth.

The patient was sent home for a month under treatment with tincture of iodine and kaolin, and advised to have his infected teeth extracted. He returned in a month complaining of only two or three movements daily, and on proctoscopic examination he was 85 per cent improved. He went home and for the next five months passed only two or three stools a day with very little blood, but in July, with an acute exacerbation, he had from eight to fifteen stools daily with much blood and pus. After some improvement, he returned home but without much relief, and at a visit in September he also had a severe myositis. In October, 1924, a colostomy was performed. Slight improvement followed, but he returned the first week in March, 1925, and was given a severe course of vaccine treatment. He was dismissed the last of the month with a permanent colostomy. He was found to be in good condition clinically and the colon was healed proctoscopically.

SPINDLE CELL SARCOMA OF THE HEART *

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AND

HARVEY S THATCHER, M D

CLEVELAND

Tumors of the heart, although observed by the early anatomists, are of uncommon occurrence. In 1685 Zollicofferus¹ wrote a dissertation on the subject, "De Polypo Cordis," and in 1700 Boneti² noted an intracardiac polyp attached by multiple roots to the wall of the right ventricle.

There are in the literature about 150 case reports of neoplasm primary in the heart, but reviews of the subject by different writers do not agree in the number that is authentic. Eighty-six³ and thirty⁴ cases, respectively, were compiled in 1893, and forty-six⁵ and ninety-one⁶ in 1908. The discrepancy in these numbers is due to the question in each recorded case whether the tumor had its origin in the heart.

As indicated by the foregoing reports the most frequent of the primary tumors of the heart are fibromas, myxomas and fibromyxomas. Rhabdomyomas, lipomas, angiomas, lymphangiomas and teratomas are rare. It is maintained by several investigators that many tumors reported as fibromas or fibromyxomas are thrombi that have become organized.

Thirty-one cases of sarcoma primary in the heart were compiled by Perlstein⁷ in 1918. To this may be added three cases that have been reported during the interim⁸.

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1 Zollicofferus, quoted by Morgagni. De Sedibus et Causis Morborum, Epist Anat Med Article 30, 24 431.

2 Boneti, Theophilus. Sepulchretum sive Anatomia Practica ex Cadaveribus Morbo Denatis, Lib IV, Sect 12, Observatio 19, p 567, Tome I, Geneva, 1700.

3 Tedeschi, A. Beitrag zum Studium der Herz-geschwulste, Prag med Wchnschr 18 121, 1893.

4 Berthenson, Leo. Zur Frage von der Diagnose primärer Neoplasmen des Herzens, Myxom des linken Vorhofes, Arch f path Anat u Physiol u f klin Med 132 390, 1893.

5 Hagedorn, Oswald. Ueber primäre Herztumor, Centralbl f allg Path u path Anat 19 825, 1908.

6 Link, Richard. Die Klinik der primären Neubildungen des Herzens, Ztschr f klin Med 67 272, 1909.

7 Perlstein, I. Sarcoma of the Heart, Am J M Sc 156 214 (Aug) 1918.

8 Gottel, L. Fall von primärem Herzsarkom, Deutsch med Wchnschr 45 1038, 1919. Goldstein, H. I. Tumors of the Heart with a Report of Ten Cases of Cardiac Tumor, New York M J 115 97, 158, 1922. Penault, L. G. A Case of Primary Sarcoma of the Heart, Canad M A J 13 108, 1923.

Neoplasms have been found in any of the four chambers of the heart originating from either endocardium, myocardium, epicardium or parietal pericardium. In the ninety-one cases tabulated by Link,⁶ the localization was as follows: right auricle, ten; left auricle, twenty-four; right ventricle, fourteen; left ventricle, eight; valve tumors, sixteen; both auricles, three; both ventricles, two; right auricle and right ventricle, two; left auricle and left ventricle, two; and interauricular septum, two.

Analyses of the reported cases by several writers have led to no definite clinical picture diagnostic of the condition. The clinical picture varies with the location and the nature of the neoplasm, and these conditions are so variable that, according to Perlstien,⁷ a neoplasm of the heart has never been diagnosed clinically. The location of the tumor may determine the competency of a valve, the integrity of the conducting mechanism or the patency of a coronary or pulmonary vessel, the size of the tumor may modify the functional condition of the myocardium or the capacity of a chamber to contain blood. A small sessile tumor may produce no symptoms, a pedunculated tumor may act like a ball-valve, and a rapidly growing tumor may lead to a very rapid disintegration of the cardiac mechanism. The contour of the heart may be distorted, and a tumor involving the epicardium may produce hemorrhage into the pericardial cavity. When present, metastases establish the diagnosis of malignancy.

In the case here reported the clinical diagnosis of sarcomatosis was made by the presence of disseminated sarcoma nodules. Involvement of the heart with sarcoma was suggested by the subnormal blood pressure, rapid pulse rate, systolic murmur, and an abnormal contour of the heart as seen in the roentgenogram. This was considered as a possibility but a definite diagnosis of sarcoma primary in the heart was not made clinically.

REPORT OF CASE

History—A Russian man, aged 27, whose general health had been good, had as the first symptoms of illness an attack of nausea, vomiting and abdominal cramps, which occurred four months before death. These symptoms disappeared after a few days. About three weeks later, the patient noticed for the first time several painful nodules developing in the legs, the arms and the buttocks. The appearance of the nodules was followed by another attack of abdominal pain and this was accompanied with diarrhea, which lasted a few days. The physician attending the patient during that attack found the abdomen distended and rigid, the temperature, 38 C, the white blood count, 4,000, the stool negative for blood, parasites and ova, and the blood Wassermann reaction negative.

The patient was admitted to Lakeside Hospital, Sept 28, 1924, with the complaint of painful nodules in the buttocks, scrotum, legs and arms.

Physical Examination—The patient was cachectic and presented marked pallor and dehydration. The temperature by mouth was 39 C. About thirty-five firm, discrete, well circumscribed nodules could be palpated in the muscles or in the subcutaneous tissue of the buttocks, scrotum, lower legs, right thigh, left upper

arm and chest. The nodules varied from a few millimeters to 1.5 cm in diameter. Several nodules in the buttocks, scrotum, and chest elevated the skin. The larger nodules were painful to palpation. There was no general glandular enlargement. The neurologic examination was negative. The lungs were negative. The respiratory rate was 22 per minute. There was no marked precordial pulsation and no thrill. The heart sounds were distinct. A soft systolic murmur was heard over

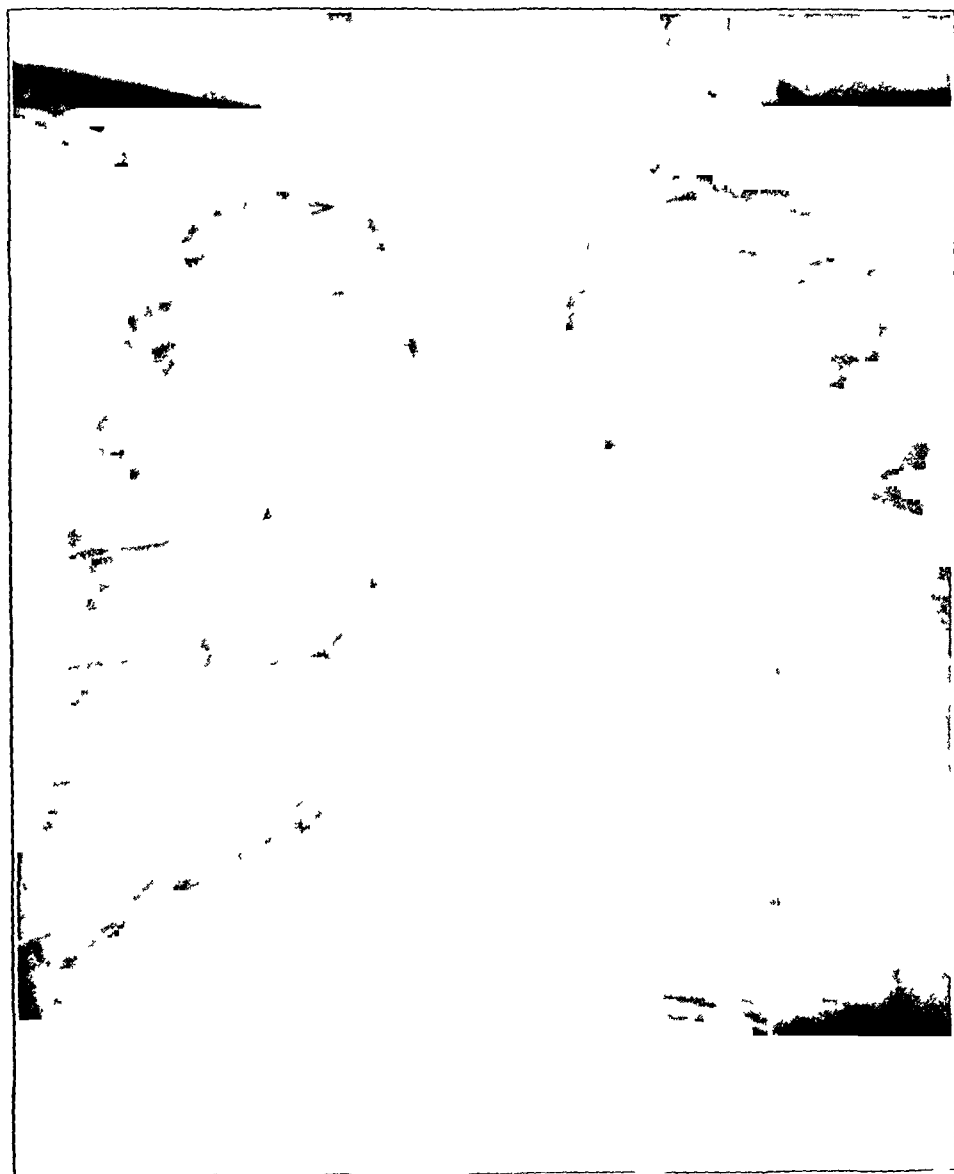


Fig 1—Chest, showing a prominence in the region of the left auricle. The cardiopericardial shadow suggested the probability of an increased amount of fluid in the pericardial cavity.

the precordium of the same intensity and quality at the base as at the apex. The first sound was clear. The systolic blood pressure was 96, the diastolic 56. The pulse rate was 120, and the rhythm was regular. The abdomen was moderately distended, tympanitic, and contained a small amount of free fluid. There was tenderness to deep palpation in the left lower quadrant. A mass of indefinite outline was palpated in the hypogastrium. A rectal examination was normal.

Laboratory Findings—The urine was normal. The blood examination showed hemoglobin, 60 per cent, red blood cells, 2,887,500, with no nucleated red cells, white blood cells, 15,400, with differential count of polymorphonuclears, 77 per cent, mononuclears, 17 per cent, transitionals, 5 per cent, neutrophil myelocytes, 1 per cent. The vital capacity was 3,750 cc in a sitting posture, the expected capacity, 4,600 cc. A blood culture was negative.



Fig 2—Section of a metastatic nodule removed from the deltoid muscle, showing spindle cell sarcoma.

A roentgenogram of the chest showed a well marked prominence in the region of the left auricle and pulmonary artery and a cardiopericardial shadow rather globular in shape, suggesting an increased amount of fluid in the pericardial cavity (Fig 1).

The nodules described above were considered metastatic and sarcomatous. A small tumor removed from the right deltoid region was a spindle cell sarcoma (Fig 2).

During the third day in the hospital the patient developed generalized peritonitis. The abdominal distention increased, the left lower quadrant became painful and rigid to palpation, and the temperature rose to 40.5 C. Death occurred on the seventh day after admission to the hospital.

The diagnosis was generalized sarcomatosis of undetermined origin, with metastases involving (1) the intestine, producing a perforation and peritonitis, and (2) the muscles and subcutaneous tissue of the legs, buttocks, etc.

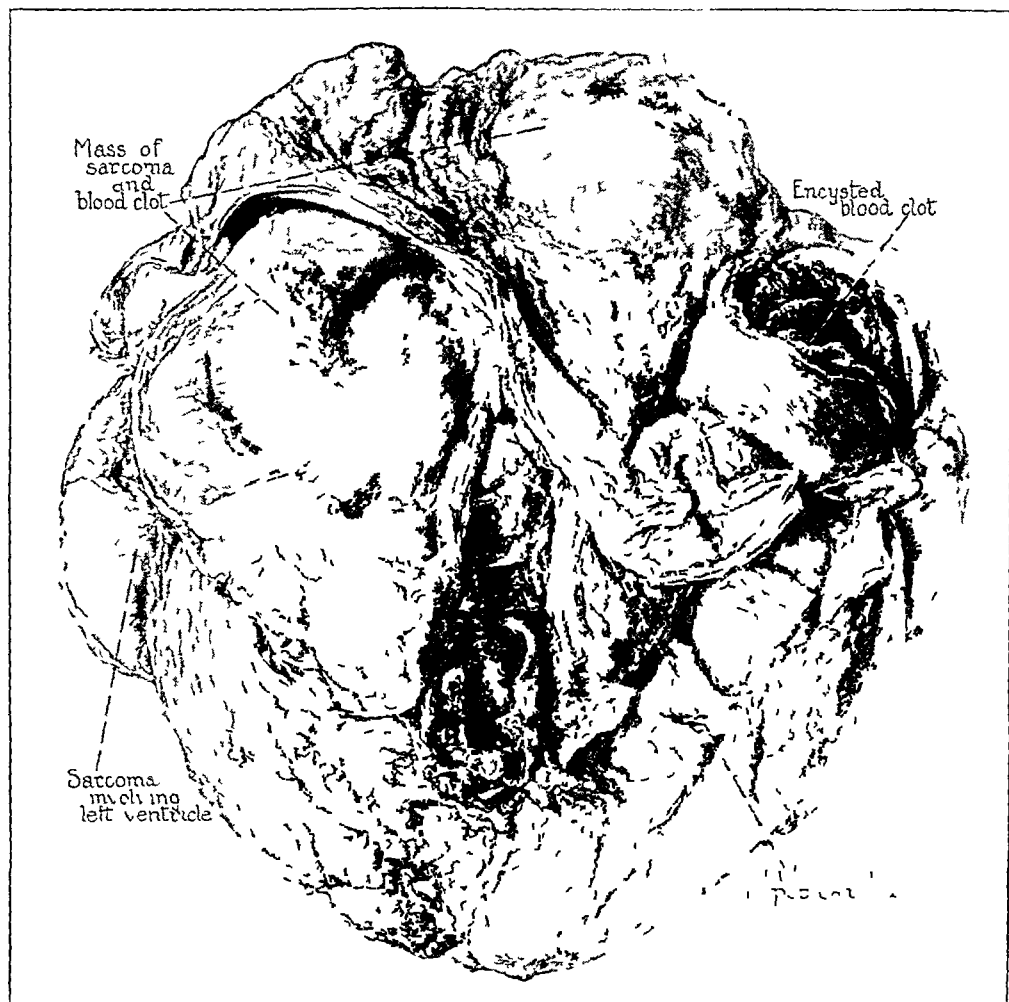


Fig 3—Posterior aspect of sarcoma with the pericardium partially removed. Multinodular mass of sarcoma and blood clot over base of heart was covered by pericardium and the latter was not perforated by the tumor. An encysted mass of blood clot is shown to the right. The pericardial cavity contained about 250 cc of free blood.

Necropsy—The metastatic nodules of the subcutaneous tissues and muscles described above were spindle cell sarcomas. There was no fluid in the chest, and no pleural adhesions were present. The pleura of the diaphragmatic, medial and posterior aspects of each lung was studded with firm, grayish white sarcomatous nodules varying in size from 0.5 to 2 mm in diameter. Sarcoma was not found elsewhere in the lungs. The heart and pericardium weighed 1,100 gm. The pericardial cavity contained about 250 cc of free blood, part of which was coagulated. Posteriorly between the heart and the pericardium was a multinodular tumor of which the greatest thickness was about 5 cm, and which extended over the entire base of the heart and partially over the ventricles (Fig 3). The

pericardium was adherent to the tumor, but its continuity was not broken. The mass in the pericardial cavity posteriorly consisted chiefly of encysted blood clot which was partially organized. The tumor at the base was an extensive sarcoma of the left auricle, several centimeters thick, displacing the pulmonary artery and its branches markedly upward, and extending through the endocardium between the entrance of the pulmonary veins. Here there were two intracavitary tumors (Fig 4). One was a pedunculated tumor, the center of which contained sarcoma, measuring about 3 by 4 cm over the surface, and about 2.5 cm to the attachment

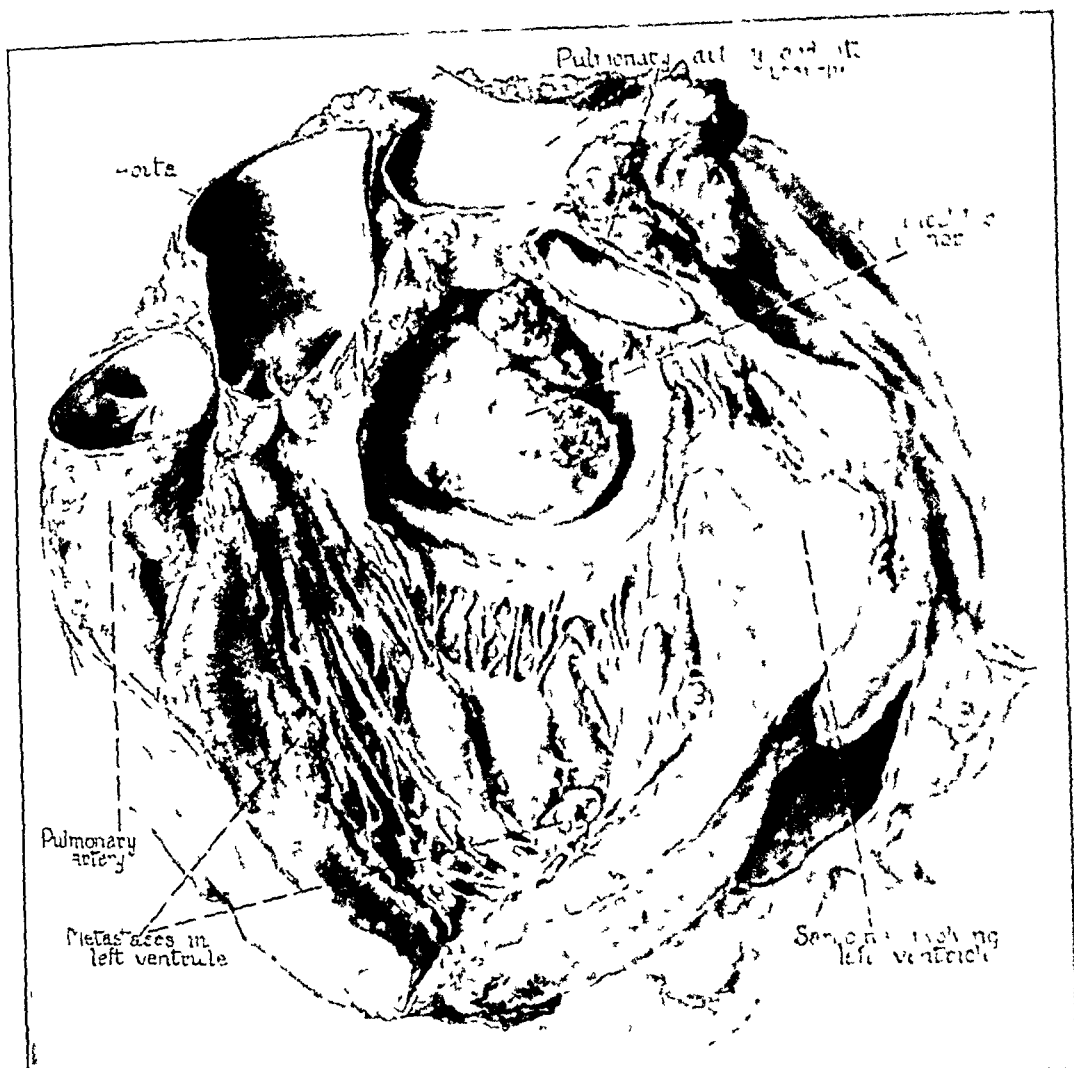


Fig 4—Extensive sarcoma involving the wall of the left auricle, displacing the pulmonary artery and its branches upward, extending into the cavity of the left auricle and into the wall of the left ventricle. Two metastases are present in the ventricle. The larger of the intracavitary tumors is a thrombus attached to the auricular wall by a pedicle that contains sarcoma. Lying above and behind the thrombus and partially obscured from view is a friable sarcoma which, undoubtedly, was the source of metastatic emboli. The pulmonary veins open on either side of these tumors and cannot be seen.

of the pedicle. It was firm, distinctly laminated, and in the outer portion consisted of dense fibrous tissue containing few nuclei. Sarcoma was present in the center of the tumor and in the pedicle. The tumor was not sufficiently free to act as a ball-valve at the mitral ring but, undoubtedly, it offered some obstruction to the filling of the left ventricle. The other intracavitary tumor was a lobulated sarcoma, about 2 cm in diameter, and partially hidden by the pedunculated tumor (Fig 5). This tumor was friable and, undoubtedly, was the source of multiple

sarcomatous emboli which were borne by the arterial blood. Anteriorly, the sarcoma extended over the left ventricle about 6 cm from the mitral ring, and involved the epicardium and the outer portion of the myocardium. Two metastases, about 0.5 cm in diameter, were present in the left ventricular wall near the septum and 3 cm from the apex. The right ventricle and the right auricle were not involved.

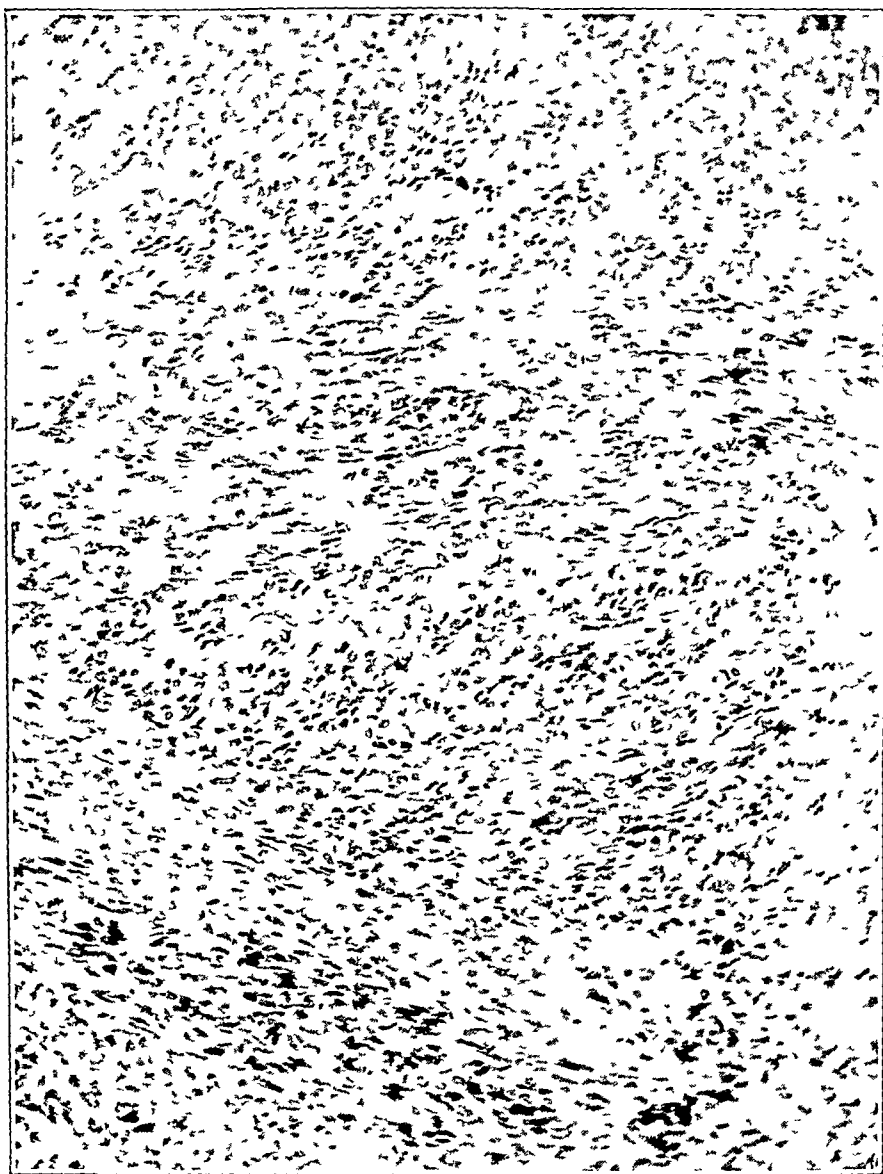


Fig 5—Section of the tumor of the heart showing spindle cell sarcoma

The abdominal cavity contained about 500 c.c. of fluid stained with fecal material and green pus. Numerous nodules, about 2 mm in diameter, were present in the parietal peritoneum. The intestines were bound together by fibrinous adhesions. A soft irregular mass, about 17 cm in its greatest dimension, was found in the left upper quadrant binding together the ileum and the transverse and descending colon. In the center of this tumor was a perforation into the ileum. Numerous tumors were found elsewhere involving the various coats of the intestines and the stomach, varying from 0.5 to 11 cm in diameter. Sarcoma was not found in the liver or brain.

COMMENT

Clinically, the presence of widely scattered nodules with the history and signs of intestinal obstruction and perforation indicated the diagnosis of sarcomatosis with metastases distributed by the arterial blood stream. Involvement of the heart was suggested by the presence of a murmur, low blood pressure, and a distortion in the cardiopericardial roentgenogram. Assuming that one pathologic condition should be held accountable for the entire clinical picture, we made the diagnosis of sarcoma involving the intestines and the muscles and subcutaneous tissues.

The primary growth was, undoubtedly, in the wall of the left auricle. The distribution of the metastases indicated that they were borne by the arterial blood stream. The absence of metastases in the liver and lungs excludes the tumors of the intestine as the primary focus. The thrombus in the left auricle formed on the sarcoma that had involved the endocardium.

QUINIDIN IN THE TREATMENT OF AURICULAR FIBRILLATION, ESTABLISHED, PAROXYSMAL AND TRANSIENT *

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In spite of the fact that quinidin has been widely used over a period of six years in the treatment of auricular fibrillation, there still exists a difference of opinion concerning its indications and contraindications, as well as its true value and dangers. It therefore seems worth while to add to the already existing reports of its use, so that more material may be available for determining our final opinion regarding the usefulness of this drug in complete arrhythmia.

TYPES OF AURICULAR FIBRILLATION

For convenience, cases of auricular fibrillation may be divided into three types—established, transient and paroxysmal.

1 Established. In this type the abnormal rhythm has been present for more than two weeks and has shown no tendency to alternate with sinus rhythm, and the presence of the irregularity is not dependent on a transient contributory factor.

2 Transient. This group includes the patients showing auricular fibrillation in association with some transient factor, in which the arrhythmia tends to disappear spontaneously after the contributory factor is no longer active.

3 Paroxysmal. Of this type are those patients who usually show sinus rhythm but who have periodic attacks of auricular fibrillation lasting from a few minutes to several days.

ESTABLISHED AURICULAR FIBRILLATION

Twenty patients selected from a total of eighty-eight with established auricular fibrillation were given quinidin. We would now exclude some of those treated, as others had been excluded, on account of evidence of serious degeneration of the ventricular myocardium. Inability to secure cooperation from some patients or their unwillingness to accept hospital admission prevented the inclusion of several more suitable patients. In no case was the drug given when well marked chronic passive congestion had recently been present, and still other patients who were deemed unsuited for this therapy were

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excluded because we doubted that their circulations would prove more efficient with sinus rhythm than with auricular fibrillation under digitalis control.

In the main, the therapeutic administration of quinidin to our established cases of auricular fibrillation covered a period of not more than one day. A test dose of 0.2 gm. or 0.4 gm. was given the first day in order to forecast if possible any unfavorable reaction to the drug, and during the day following, 2.4 gm. or less was administered in increments of 0.4 gm. It has been shown experimentally that quinidin is a cardiac poison, producing, in high concentration, ventricular tachycardia, difficulties in conduction, and finally ventricular fibrillation.¹ Moreover, various clinical observers have reported disturbing or serious results when this drug had been given in considerable dosage over a period of days. Sudden deaths have occurred, some due to embolism,² but others not clearly due to this cause.³ These untoward results suggest caution.

The economic factor involved in quinidin therapy also contributed considerably to our decision to use this plan of treatment. Even after sinus rhythm is reestablished, practically all these patients relapse sooner or later into auricular fibrillation, and must be retreated with quinidin if it is desired to maintain regular rhythm. These repeated quinidin treatments necessitate hospital admissions since it is quite possible for unfavorable symptoms to arise in a second course (Case 1), even though the original experience has gone smoothly. However, we found that the difficulty or ease with which auricular fibrillation is interrupted by quinidin on the original treatment is apt to represent a fair forecast of the readiness with which regular rhythm may be again secured after relapse has occurred. And therefore it seems that if the original treatments depend for success on several days of high concentration of quinidin, then there will be a greater incidence of serious or disturbing reactions, and less likelihood of results that would prove of any lasting benefit to the patients.

All who were given quinidin had previously received sufficient digitalis to slow the ventricular rate below 90 per minute, the digitalis then being discontinued.

In those patients who could be successfully followed after the establishment of regular rhythm, quinidin was usually administered daily for a few weeks in doses of from 200 to 600 mg. in order to prevent an

¹ Korns, H. M. Experimental and Clinical Study of Quinidin Sulphate, *Arch. Int. Med.* **31** 15-35 (Jan.) 1923.

² Wyckoff, J., and Ginsberg, M. *Boston M. & S. J.* **190** 771-776 (May 8) 1924. Levine, S. A., and Wilmaers, Albert. *Boston M. & S. J.* **192** 388-392 (Feb. 26) 1925.

³ Levy, R. L. Clinical Toxicology of Quinidin, *I. A. M. A.* **30** 1108-1113 (Sept. 30) 1922.

early recurrence of the irregularity After this, on account of the fact that in many cases regular rhythm may be maintained unaided for long periods, the patients were permitted to do without the drug unless relapses occurred Quinidin was retried in five cases and was successful in each instance

Really satisfactory results, in which the patients were able to maintain a regular rhythm for long periods with apparently improved comfort and general health, except for brief lapses into auricular fibrillation, were obtained in four cases, or 20 per cent

Regular rhythm was established in 55 per cent of the entire group, and if the two patients are eliminated who responded unfavorably to the test dose the percentage in which regular rhythm was established was 61

An attempt to analyze too minutely a short series of cases may lead one into error, but certain features of this short term plan of administering quinidin in twenty established cases of auricular fibrillation as evidenced in the accompanying table seem worthy of comment

The cases were equally divided between the arteriosclerotic, the senile or the degenerative type, and those in which mitral disease was the main contributing factor, there being ten of each In the mitral disease group regular rhythm was established in 70 per cent, while in the degenerative group only 40 per cent became regular

The average amount of drug necessary to bring about sinus rhythm in the mitral group was 1,142 mg, while in the degenerative group it was 1,450 mg

The average duration of regular rhythm in the mitral group was ninety days, while in the degenerative group it was only twenty days

Judging from the histories obtained from all the patients, the average length of time during which auricular fibrillation had been present in those who became regular after quinidin was five months, while in those who did not respond, the average duration had been nineteen months

ELECTROCARDIOGRAPHIC CHANGES

Among the patients who regained normal rhythm the auricular waves were registered as coarse or moderately coarse in 75 per cent, but in only 33 per cent of those in whom fibrillation persisted Among the nine cases in which there was no response to quinidin were two cases of intraventricular block and one of right bundle branch block After sinus rhythm had been established, two patients showed extrasystoles, one of auricular, and the other of nodal origin

UNTOWARD SYMPTOMS

In two cases no more than a test dose was given on account of unfavorable response Six patients were considerably nauseated, vomit-

Results of Treatment with Quinidin

	History of Duration of Fibrillation	Reaction to Test Dose	Number of Days Treated with Quinidin	Drug Discontinued Because of Unfavorable Symptoms	Total Amount of Drug Given, Gm	Nausea	Vomiting	Diarrhea	Dyspnea	Increased Cyanosis	Rapid Ventricular Rate	Paroxysmal Atrial Fibrillation	Regular Rhythm	Duration of Regular Rhythm	Quinidin Retried After Relapse	Duration of Regular Rhythm on Retry	Improved General Health	Relative Cardiac Buliness, Cm	Comment	
																		Left Right		
1	2 mo	None	1	0	20	0	0	0	0	0	0	0	+	5 mo	3 times	17, 6, 9 mo	++	16	< 3	Dyspnea, cyanosis, paroxysmal tachycardia and collapse following second treatment
2	Not known	None	1	0	0.1	0	0	0	+	+	+	+	+	1 hr	0	0	0	10	< 3	
3	3 mo	None	1	+	16	+	0	+	0	0	0	0	+	1 mo	0	0	0	11	< 1	Patient unwilling to enter hospital for second treatment
4	3 mo	None	1	0	0.1	0	0	0	0	0	0	0	0	1 mo	+	Not known	?	11	< 1	Relapse was of paroxysmal type
5	3 mo	None	1	0	0.1	0	0	0	0	0	0	0	0	6 days	0	0	0	12	< 1	Quinidin not tried on account of lack of cooperation of the patient
6	2 mo	None	1	0	2.1	0	0	+	0	0	0	0	0	0	0	0	0	10 1/2	< 3	
7	3 yr	None	1	0	1.2	+	0	0	0	0	1.20	0	+	8 days	+	Not known	?	11	< 1	Patient went to South America
8	1 mo	None	1	0	16	0	0	0	0	0	0	0	0	11 mo	0	0	-	14	< 1	Still regular
9	Not known	None	1	+	1.2	+	0	+	0	0	0	0	0	0	0	0	0	15	< 1	
10	1 mo	None	1	0	2.1	0	0	0	0	0	0	0	0	0	0	0	0	11 1/2	< 1	Sudden death 29 hours after last dose of quinidin
												</								

ing occurred in one, diarrhea in three, dyspnea in five, increased cyanosis in five, dizziness in two, the ventricular rate rose above 120 in four, paroxysmal auricular tachycardia occurred in two, and collapse in one. In the following case, death occurred twenty-nine hours after quinidin had been discontinued, the heart having failed to become regular. This patient had mitral disease and a large heart.

REPORT OF CASE

CASE 1—A woman, aged 22, was admitted to the hospital, Feb 2, 1923, complaining of rapid heart action. She had had diphtheria at eight years and rheumatic fever at the age of twenty. Tonsillectomy had been done three years previous to the rheumatic attack.

The onset of the present illness followed rheumatic fever two years before her admission. There was then some palpitation for a month or so with subsequent improvement. Rapid and irregular heart action had been present for the last month.

There was slight cyanosis of the lips but no dyspnea or edema. The tonsillar tissue had recurred on the left, and the thyroid gland showed some diffuse enlargement. The cardiac dulness was considerably enlarged to the right and the left, the relative dulness measuring 14.5 cm to the left of the midsternal line in the fifth interspace and 4 cm to the right in the fourth. The heart's action was completely irregular. The apex rate was 140 per minute and the radial count was 110. There was a harsh, blowing systolic murmur at the apex. The second pulmonic sound was accented. The liver edge was at the costal margin, there were no râles at the bases of the lungs, and there was no edema of the lower extremities.

The patient was given 4 drams (15 cc) of tincture of digitalis over a period of three days, the pulse deficit disappearing and the apex rate slowing down to 70 per minute, though still completely irregular.

On the fourth day, a test dose of 0.4 gm of quinidin was given without any reaction. The day following 2 gm was administered in increments of 0.4 gm. The cardiac action continued irregular and on the next day was 90 per minute. No disturbing gastro-intestinal symptoms were noted. At 9:15 p. m., twenty-nine hours after the last dose of quinidin, the patient remarked to the nurse who was in the room how well she felt, when suddenly her facial muscles began to twitch, she rapidly became cyanotic and dyspneic, and died in a few moments. On the last observation, shortly before the patient's sudden death, the pulse had been completely irregular. A necropsy could not be secured.

It does not seem possible to draw a clear conclusion as to the cause of death in this case. It may be said that it should not necessarily be charged to quinidin. Lewis⁴ has shown that quinidin is very quickly destroyed in the body, and in twenty-four hours is apt to be largely eliminated. At the end of this period it is doubtful whether regular rhythm and resulting cerebral embolism would ensue from quinidin if it has not occurred during the higher concentration of the drug administered the preceding day. The death may have been due to cerebral embolism unrelated to quinidin administration. However, it seems difficult to escape the probability that there was some relation to the drug therapy.

⁴ Lewis T. *Am J M Sc* 163 781 (June) 1922

TRANSIENT AURICULAR FIBRILLATION

Instances of transient auricular fibrillation, according to our definition, have been so frequent in our experience that we are not inclined to include the detailed case reports. However, the principal contributing factors, in the order of their frequency, have been

- 1 Thyroidectomy
- 2 Toxic adenoma of the thyroid
- 3 Major surgical operations, such as cholecystectomy
- 4 Acute lobar pneumonia

5 Certain miscellaneous cases, which have included exhaustion from extreme overexertion, acute alcoholism, starvation and dehydration in association with acute cholecystitis

As is well known, auricular fibrillation develops frequently after thyroidectomy. It commonly occurs the first or second day after operation and constitutes a disturbing but not necessarily serious complication. Its occurrence is an indication for digitalization of the patient, a procedure that is generally sufficient to increase his comfort, the irregularity of the pulse disappearing spontaneously in twenty-four or forty-eight hours. In cases in which the arrhythmia persists on into convalescence, it is, as a rule, easy to produce a reversion of sinus rhythm with quinidin.

Toxic adenoma of the thyroid, occurring as it does largely in people over 50 years of age, is also a potent factor in the production of auricular fibrillation. The classification of the arrhythmia in these instances as transient, even when the irregularity has been present for long periods—months or years—may be questioned. It is true that the arrhythmia does persist after thyroidectomy in some of these patients, but, at least before operation, the auricular fibrillation should always be thought of as potentially transient. In the majority of our patients with hyperthyroidism and auricular fibrillation who have had thyroidectomy, the arrhythmia has disappeared spontaneously during convalescence, without any need for quinidin therapy. Our experience in this regard is in accord with that of Dunhill, Fraser and Stott,⁵ who likewise noted a spontaneous resumption of normal rhythm in thyrotoxic auricular fibrillation after removal of the thyroid in a considerable proportion of the cases.

It would seem that in transient auricular fibrillation, quinidin, although of occasional value, is seldom indicated, since the tendency is for spontaneous resumption of normal rhythm after the elimination of the transient factor.

⁵ Dunhill, T. P., Fraser, F. R., and Stott, A. W. *Quart J Med* **17**: 326-338 (July) 1924.

PAROXYSMAL AURICULAR FIBRILLATION

Instances of paroxysmal auricular fibrillation, or flutter, associated with hyperthyroidism are excluded from this report, since they are best treated by thyroidectomy

CASE 2—A university professor, aged 68, for six years had been subject to attacks of auricular fibrillation at intervals of a month or more. In the last year the attacks had been occurring more frequently, at intervals of every two weeks. The physical examination showed no remarkable cardiac findings. The blood pressure was 144 systolic and 80 diastolic. There was no marked evidence of arteriosclerosis and no obvious cardiac hypertrophy. An electrocardiogram was made during the period of regular rhythm and showed no abnormality.

This patient began taking quinidin sulphate, 200 mg daily, and continued this for six weeks. During that time he did not feel that there was any decrease in the frequency of his attacks or any shortening of the attacks that did occur.

CASE 3—An Arabian woman, aged 50, who was admitted to the hospital, March 7, 1925, stated that for five years she had been subject to spells of palpitation, or heart fluttering. During the last month, these attacks had been especially frequent, coming on every four or five days and being aggravated by exertion.

The patient was a very obese woman. The cardiac rhythm was completely irregular, the rate was 150. The pulse rate was 80. During the examination, the rhythm suddenly became regular and the patient announced that she felt better again. An electrocardiogram made during the period of regular rhythm showed no abnormality.

The patient was placed on quinidin sulphate, 400 mg twice a day, and this was in a short time reduced to 200 mg twice daily. Under this regimen the frequency of the attacks was diminished from every few days to every two weeks, and the length of the attacks diminished from an average of from four to ten hours to an average of one-half hour.

CASE 3—A man, aged 50, who had been followed since April, 1920, had been subject to periodic attacks of palpitation since February, 1920. The personal history was unimportant.

The blood pressure was 145 systolic and 80 diastolic. The relative cardiac dulness extended from 10.75 cm to the left in the fifth interspace to 3 cm to the right in the fourth interspace. The heart action was unusually regular. There was a soft systolic murmur at the apex and also in the aortic area. An electrocardiogram showed evidence of incomplete bundle branch block.

He had been observed repeatedly during paroxysms of auricular fibrillation. These had occurred at irregular intervals, seldom not more frequently than once a month, and often he had freedom from paroxysms for six months. The average duration of the attacks had been from six to ten hours. During an attack the administration of 400 mg of quinidin was tried but it seemed to increase the palpitation and did not serve to terminate the attack in a shorter time than usual. In the majority of the paroxysms, he had been given tincture of digitalis, 1½ drams (5.6 cc), and this had served to diminish the palpitation within an hour or two and seemed to shorten the attacks.

CASE 4—A woman teacher, aged 52, was admitted to the hospital, Nov. 10, 1923, complaining of heart trouble. There was a previous history of acute rheumatic fever, tonsillitis and scarlet fever as a child.

The patient had known that she had some heart trouble for the last ten years. One year before admission, she developed attacks of rapid and irregular heart action, which occurred with great frequency and sometimes lasted for twenty-four hours. These attacks had been brought on by eating or by any exertion.

On examination she proved to be considerably undernourished. The cardiac apex impulse was felt in the sixth interspace, 7 cm to the left of the midsternal line. The cardiac dulness extended 7 cm to the left in the fifth interspace and 4 cm to the right in the fourth interspace. The first sound at the apex was very

snapping There was a blowing systolic murmur at the apex and also a faint presystolic rumbling murmur

During the periods of regular rhythm, the electrocardiogram showed some T wave flattening and auricular extrasystoles The P waves in Lead II were somewhat enlarged Auricular fibrillation developed frequently after the eating of a meal, and was observed to be brought on after exercise

This patient was given quinidin sulphate, 200 mg twice daily Two days after this was begun, the heart was again observed to be irregular and the drug was increased to 600 mg daily, in increments of 200 mg, and this was sufficient to maintain normal rhythm This patient has continued to take quinidin daily with the most remarkable improvement in her comfort and health Although she had previously been completely disabled from teaching, she returned to her work after suitable convalescence and has continued at it ever since In her own words, she has been on "full time work with a very heavy schedule" There have been only two very brief attacks of palpitation during a term

It is interesting that over a short period of time this patient was given quinidin through a pharmacist's mistake, and suffered a considerable increase in the frequency of the attacks This was corrected as soon as the error was detected and quinidin was again brought into use

No suggestive evidence of thyroid disease was observed in any of these patients

In paroxysmal auricular fibrillation not due to hyperthyroidism, quinidin is occasionally useful in relieving the patient of disagreeable or annoying palpitation, and this may be the only favorable effect But in some cases the disability that has resulted from very frequent paroxysms may be quite severe, as in Case 4 so that the drug may at times succeed in restoring to useful occupation patients who would otherwise have greatly diminished working efficiency

It would seem that the prophylactic use of quinidin is not necessary unless the paroxysms occur more frequently than every two weeks We are not much inclined to use the drug in the treatment of individual paroxysms unless they tend to be prolonged and then only after preliminary digitalization

Our most satisfactory results have been secured in patients who had had frequent attacks In these, the prophylactic use of from 0.2 to 0.8 gm of quinidin daily increased the period of freedom from attacks and considerably shortened the length of the paroxysms that did occur The absence of this effect in Case 2 was probably due to insufficient dosage

SUMMARY AND CONCLUSIONS

Established Auricular Fibrillation—1 We have had 61 per cent success in the production of normal rhythm from auricular fibrillation by a one day plan of quinidin administration This compares favorably with the results obtained in a large number of cases already reported (56 per cent) ⁶

⁶ Burwell, C S, and Dieuaide, F R Clinical Experience with Quinidin, Arch Int Med 31 518-525 (April) 1923

2 The patient with mitral disease who has had the arrhythmia for a relatively short time is most likely to revert to normal rhythm after quinidin, and in such patients the resulting regular rhythm will likely persist longer than in the arteriosclerotic or degenerative type

3 Patients of the arteriosclerotic or degenerative type who have been irregular for long periods are often more difficult to restore to normal rhythm by quinidin, and such rhythm if produced is apt to be of relatively short duration

4 Patients with coarse electrocardiographic auricular waves are more easily rendered regular by quinidin than those who show fine auricular waves

5 One death occurred in association with quinidin therapy, in our patients

6 In the mitral disease group, quinidin should be used only by those patients without marked cardiac hypertrophy and dilatation who have had the irregularity for but a short time and who have not had congestive cardiac failure

7 In the degenerative group, we are inclined to believe that the use of quinidin is not justified

8 In no case of established auricular fibrillation should this drug be used unless it is apparent that close cooperation is going to be possible between the patient and the physician, and unless hospital admissions and readmissions can be readily arranged if thought necessary

Transient Auricular Fibrillation—Quinidin was found indicated only when the arrhythmia persisted after the disappearance of the transient causative factor. In cases associated with hyperthyroidism quinidin apparently should not be used until six weeks after thyroidectomy

Paroxysmal Auricular Fibrillation—1 In those cases not due to hyperthyroidism in which the paroxysms were frequent, quinidin administered prophylactically, in doses of from 0.2 to 0.4 or 0.8 gm daily, was found to be of the greatest value in lengthening the interval between the attacks and in shortening the paroxysms that did occur

2 In the treatment of individual paroxysms, quinidin need be used only in rare instances when the paroxysm tends to be prolonged

3 Quinidin was not found necessary in daily dosage unless the paroxysms occurred more often than once in two weeks

The chief value of quinidin was found in the treatment of frequent paroxysmal auricular fibrillation when not associated with hyperthyroidism. It has many dangers and an extremely limited field of usefulness in the established cases, and real benefit of any considerable duration is derived in only a small proportion of these

THE VALUE OF THE ICTERUS INDEX IN DIFFERENTIATING ANEMIA

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Everyone realizes the pitfalls and difficulties that all except the most highly trained hematologists encounter in the differentiation of the anemias. Mistakes in diagnosis are not uncommon, especially is this true when the busy clinician mistakes a severe secondary anemia, such as may occur in connection with carcinoma, for a case of pernicious anemia¹. In just such types of cases anything that may aid in making a correct diagnosis simply and rapidly is worthy of consideration. Our aim, therefore, was to determine what clinical value, if any, the icterus index might have in this direction.

We have interested ourselves primarily with a clinical fact, namely, the intensity of the yellow color of the blood serum, and in this study have not concerned ourselves particularly with the numerous considerations as to the possible sources of this color.

As this was to be a clinical test, the simplest and most rapid means of determining the icterus index was desired. Hence, we made use of a modification of Meulengracht's bilirubin colorimeter². For his standard he uses 0.05 parts of potassium dichromate in 500 c.c. of distilled water, with 2 drops of sulphuric acid, this gives a depth of color corresponding to an icterus index of 1. He then determined the bilirubin content of the blood as shown by the amount of physiologic sodium chlorid solution that has to be added to the plasma to bring the tint to correspond with the standard. By our modification, using the same standard solution for an index of 1 and having a series of permanent standards of greater intensity of color, the color comparison is made directly, this does away with the necessity of diluting the specimen in a manner similar to that done by the Dunning apparatus of phenol-sulphonephthalein estimation in the urine.

It is understood that the method of Hymans-Van den Bergh³ for the quantitative estimation of bilirubin in the serum is probably slightly more accurate, but we believe that this added accuracy is clinically

* From the Department of Pathology of Bellevue Hospital

¹ St George, A V. Proc New York Path Soc **22** 6-8, 1922

² Meulengracht, E. Deutsch Arch f klin Med **137** 38 (Aug) 1921

³ Van den Bergh, A A, Hymans, and Snapper. Deutsch Arch f klin Med **110** 540, 1913

unnecessary and that, for ordinary clinical purposes, the test is needlessly complex of performance so far as the differentiation of the anemias is concerned

In order to obtain our own figures for the normal range of the icterus index, the determination was made on the plasma of 120 consecutive nonprofessional blood donors, ranging in age from 18 to 47 years. These donors were relatives or friends of patients in the wards of Bellevue Hospital who were in need of transfusions, and the donors, as far as they themselves knew and to all outward appearances, formed a group of average healthy adults.

From Table 1 and the accompanying chart it will be noted, first, that the average index was 4.41, and, second, that 90.8 per cent of the control cases had an index between 3.5 and 5.5, inclusive. Van den Bergh³

TABLE 1—Icterus Index Determinations in One Hundred and Twenty Controls, Average Index, 4.41

Icterus Index	Number of Cases
2.0	1
2.5	0
3.0	4
3.5	15
4.0	29
4.5	38
5.0	19
5.5	8
6.0	3
6.5	0
7.0	2
7.5	0
8.0	0
8.5	0
9.0	1

obtained similar figures. This suggests a rather well defined range of normal variation, and when a determination is obtained that is either above or below this range, a clinical cause therefore should be sought. It is altogether probable, had we been fortunate enough to be able to make a thorough examination of the donors with indexes outside this range, that in the majority of cases the cause of the variation from the so-called normal could have been determined. Thus, we found that the lone index of 9 belonged to a rather obese Italian woman, aged 44, giving a typical history of chronic cholecystitis.

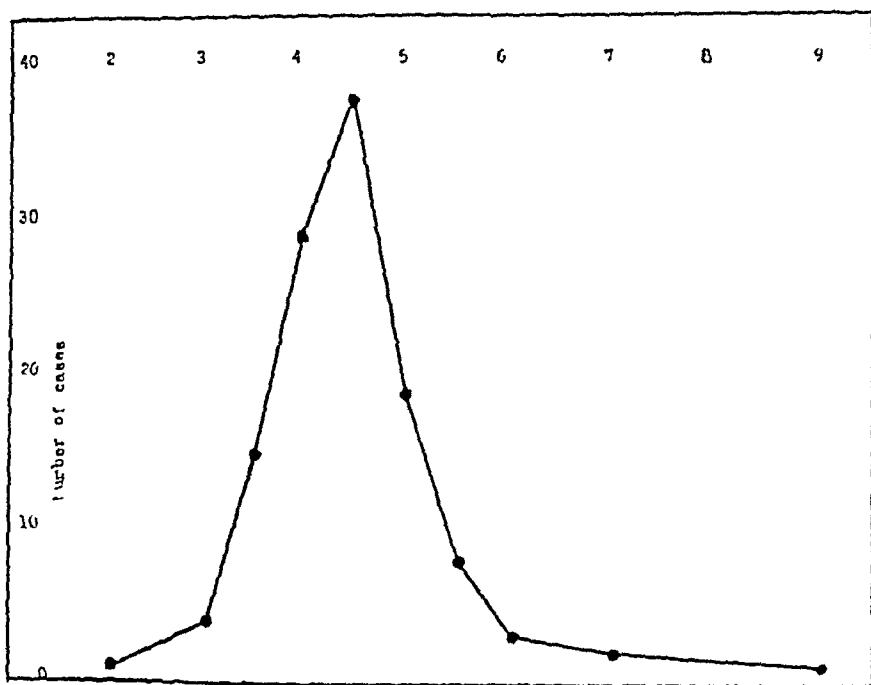
Our own series of clinical cases includes twenty-three cases of pernicious anemia, twenty-three cases of carcinoma with anemia, fourteen examples of secondary anemia and eighteen conditions other than pernicious anemia giving a high index.

Let us consider, first, the cases of pernicious anemia (Table 2). This series was selected for the reason that it includes only those in which the final diagnosis was confirmed beyond a reasonable doubt. We were fortunate enough subsequently to confirm two of these diagnoses by postmortem examination. The results are very consistent, the icterus

index in every case being well above the range of normal variation. In uncomplicated cases the range varied from 9 to 12.5, with an average index of 9.7.

The fact that we found an increased index in pernicious anemia is in accord with the work of other investigators. Andrewes⁴ presents a series of such cases, all of which gave a Van den Bergh indirect test above the normal range. Hirschfeld⁵ and Lepehne⁶ draw similar conclusions.

By complicated cases we mean those in which some factor other than hemolytic anemia is working to give a high icterus index, as in Cases 30, 34 and 36 in which the cardiac failure produced more or less



Range of icterus index in 120 control cases, average icterus index, 4.41, 90.8 per cent of all between index of 3.5 and 5.5.

marked hepatic obstruction. If, perchance, the question arises as to whether the high index in a particular case is due to hemolytic anemia or to hepatic obstruction, then Van den Bergh's direct test may be performed. (The serum from cases of obstructive jaundice gives a direct reaction, and normal serums or those from hemolytic anemias give the indirect reaction only.)

No constant relationship was noted between the height of the index and the severity of the anemia. This seems to be dependent, rather, on the phase of the cycle during which blood is taken from the patient suffering from pernicious anemia. Thus, during a bone marrow crisis

⁴ Andrewes, C. H. *Quart J Med* **18** 69, 1924.

⁵ Hirschfeld. *Ztschr f klin Med* **87** 1, 1919.

⁶ Lepehne, G. *Deutsch Arch f klin Med* **135** 79 (Jan) 1921.

TABLE 2—Icterus Index in Pernicious Anemia

Case Number	First Diagnosis	Final Diagnosis	Kind of Diagnosis	Icterus Index	Jaundice	Sex*	Age	Hemorrhage	Red Blood Cells per Cent	Hemo-globin, per Cent	Remarks
1	Pernicious anemia	Pernicious anemia	Clinical	10.0	0	♂	32	0	1,600,000	47	Blood picture typical
2	Acute cerebri-itis?	Pernicious anemia	Clinical	8.5	0	♀		0	3,100,000	56	
3	Pernicious anemia	Pernicious anemia	Clinical	9.0	It in Lemon	♀		0	2,500,000-1,200,000	45	
4	Pernicious anemia	Pernicious anemia	Clinical	9.0	Lemon	♂		0	1,200,000	40	
5	Pernicious anemia	Pernicious anemia	Clinical	9.0	0	♂		0	2,500,000-3,600,000	60	
6	Pernicious anemia	Pernicious anemia	Clinical	9.0	0	♂	55	0	1,500,000	40	
7	Pernicious anemia	Pernicious anemia	Clinical	11.5	±	♀	47	0	410,000	20	
8	Pernicious anemia	Pernicious anemia	Necropsy	8+	0	♂		0	1,500,000-2,000,000	30-80 D	
30	Pernicious anemia	Pernicious anemia	Clinical	45.0	++	♂		0	550,000	20	Cardiac decompensation +
31	Pernicious anemia	Pernicious anemia	Clinical	12.5	Lemon	♂	67	0	1,440,000	32	
32	Pernicious anemia	Pernicious anemia	Clinical	8.5	Lemon	♀	60	0	1,350,000	40	
33	Hemolytic jaundice	Pernicious anemia	Clinical	9.5	Pallo	♀	40	0	1,808,000	45	Liver and spleen +
34	Secondary anemia	Pernicious anemia	Clinical	16.0	+	♀	50	0	900,000	30	Cardiac decompensation +
35	Pernicious anemia	Pernicious anemia	Necropsy	11.5	Lemon	♀	25	0	720,000	25	Chronic cardiovascular disease
36	Pernicious anemia	Pernicious anemia	Clinical	14.0	Lemon	♂	65	0	900,000	35	
37	Pernicious anemia	Pernicious anemia	Clinical	9.0	Lemon	♀	70	0	770,000	30	
38	Pernicious anemia	Pernicious anemia	Clinical	11.0	±	♀	49	0	1,700,000	60	
39	Pernicious anemia	Pernicious anemia	Clinical	8.0	0	♀	39	0	1,130,000	25	
40	Ovarianoma (?) or pernici-ous anemia	Pernicious anemia	Clinical	12.5	±	♀	54	0	3,000,000	60	
71	Pernicious anemia	Pernicious anemia	Clinical	11.5	Lemon	♂	56	0	1,400,000	45	Splenectomy 1 year
72	Pernicious anemia	Pernicious anemia	Clinical	8.5	±	♂	40	0	2,000,000	50	Negative gastrointes-tinal roentgen-ray ex-amination
73	Carcinoma of stomach	Pernicious anemia	Clinical	9.0	Lemon	♂	48	0	1,600,000	37	Ulcerative colitis
74	Pernicious anemia	Pernicious anemia	Clinical	8.5	Pallo	♀	58	Slight	2,100,000	60	

* In this and the following tables ♂ indicates male, ♀ female

or even before the crisis, the index mounts rapidly day by day, and, similarly, before obvious clinical improvement the index gradually falls toward the normal. This suggests a clinical means of determining when it would be advisable to transfuse a pernicious anemia patient, as well as when the patient might improve spontaneously.

Table 3 consists of twenty-three cases of carcinoma with anemia. The results here also were uniformly consistent in that the icterus index was invariably at or below the normal range. Over half of these cases proved to be carcinoma on histologic examination of the new growth. In the other cases, the growth was so far advanced as to be declared inoperable.

It is well known that anemia due to an obscure new growth and pernicious anemia not infrequently present confusingly similar blood pictures to the average laboratory man, to say nothing of the busy practitioner. Similarly, as shown by Warfield,⁷ chronic infections often give a picture that on cursory examination is diagnosed as pernicious anemia. Cases 93 to 97, inclusive, are examples of this condition. In Case 97, the diagnosis made at necropsy differed from that made during life, which was pernicious anemia. The patient was a man aged 45, who had been ill for about six weeks. He was jaundiced, the liver and spleen were enlarged, the red cells totaled 610,000 and hemoglobin 15 per cent, with all forms of abnormal red cells, the Wassermann reaction was +++ repeatedly. At the time we saw the patient, the following note was made, on the basis of the icterus index, which was 46:

The height of this index cannot be accounted for by a simple case of pernicious anemia nor by uncomplicated Banti's disease. However, either of the above, plus cardiac decompensation, producing obstruction (passive congestion) in the liver, could give such an index. Again, if the anemia is the sole cause, there would have to be a tremendous amount of active blood destruction as occasionally occurs in acute arsphenamin poisoning.

In the light of our present knowledge, we could also have considered the possibility of chronic sepsis. The necropsy note by Dr Douglas Symmers contains the following statement: "At autopsy, the whole picture appeared to be easily explained on the basis of chronic sepsis with hemolytic jaundice."

This brings forth the possibility of the facile use of the icterus index as an aid in the differential diagnosis, not only of cases of hemolytic anemia from secondary anemia, but more especially of cases of pernicious anemia from anemia due to carcinoma. Thus, if we glance at Tables 2 and 3, we note that, irrespective of the degree of the anemia, the icterus index in cases of pernicious anemia was uniformly above the

⁷ Warfield, L. M. New York State J. Med. **25** 147 (Feb. 6) 1925.

TABLE 3—*Icterus Ind. v in Carcinoma with Anemia*

Case Number	Final Diagnosis	Final Diagnosis	Kind of Diagnosis	Icterus Index	Jaundice	Sex	Age	Hemoglobin	Red Blood Cells	Hemo globin, per Cent	Remarks
9	Carcinoma of stomach	Carcinoma of stomach	Operation	1.5	0	♀	42	0	3,820,000	60	Pathologic specimen
10	Carcinoma of breast	Carcinoma of breast	Clinical	3.0	0	♀		0	4,005,000	55	Inoperable
11	Carcinoma of breast	Carcinoma of breast	Clinical	1.6	0	♀	45	Slight + breast	3,840,000	50	Inoperable
12	Carcinoma of prostate, metastases to liver	Carcinoma of prostate, metastases to liver	Clinical	2.5	0	♂	71	0	3,200,000	65	Proctoscopy
13	Carcinoma of rectum	Carcinoma of rectum	Clinical	2.0	0	♂	46	++	3,100,000	70	Proctoscopy
14	Carcinoma of rectum	Carcinoma of rectum	Clinical	3.0	0	♀	44	0	3,500,000	40	Proctoscopy
15	Carcinoma of stomach	Carcinoma of stomach	Clinical	2.5	0	♀	63	0			Inoperable
16	Carcinoma of penis, chest	Carcinoma of penis, chest	Operation	3.0	0	♂	51	0	3,450,000	60	Pathologic specimen
17	Carcinoma of stomach	Carcinoma of stomach	Clinical	2.0	0	♀	61	++	2,000,000		Roentgen ray examination
18	Carcinoma of stomach	Carcinoma of stomach	Operation	3.0	0	♂		0	4,500,000	90	Pathologic specimen
19	Carcinoma of sigmoid	Carcinoma of sigmoid	Neopsy	11.0	0	♂		0	2,240,000		Pneumonia
20	Carcinoma of ulcer of stomach	Carcinoma of stomach	Operation	2.5	0	♂	47	0	4,400,000	65	Preoperative diagnosis by icterus index, carcinoma, clinically inoperable
21	Carcinoma of stomach	Carcinoma of stomach	Clinical	1.5	0	♀	61		2,000,000	33	Roentgen ray examination
22	Carcinoma of sigmoid	Carcinoma of sigmoid	Operation	2.5	0	♂	55	0	3,100,000	40	Inoperable by exploration
23	Carcinoma of sigmoid	Carcinoma of sigmoid	Operation	2.5	0	♂	60	Slight repeated	3,080,000	65	Pathologic specimen
24	Carcinoma of sigmoid	Carcinoma of sigmoid	Operation	2.0	0	♀	53	Repeated	3,740,000	70	Inoperable—colostomy
25	Carcinoma of rectum	Carcinoma of rectum	Operation and biopsy	2.0	0	♂	66	Repeated	3,770,000	65	Pathologic specimen—in operable
26	Carcinoma of larynx	Carcinoma of larynx	Operation	2.0	0	♂	53	0	3,670,000	65	Operation, died
27	Carcinoma of rectum	Carcinoma of rectum	Biopsy	1.5	0	♀	65	++	2,460,000	40	Biopsy
28	Carcinoma of esophagus	Carcinoma of esophagus	Operation	2.0	0	♂	54	0	3,110,000	40	Operation, pathologic specimen
29	Carcinoma of tongue	Carcinoma of tongue	Operation	3.0	0	♂	59	0	1,100,000	75	Biopsy
66	Carcinoma of breast	Carcinoma of breast	Clinical	2.0	0	♀	50	0	2,740,000	40	Inoperable
67	Carcinoma of stomach	Carcinoma of stomach	Operation	1.5	0	♂	54	0			Pathologic specimen see secondary anemia

TABLE 4—*Various Conditions Exhibiting a Low Icterus Index*

Case Number	First Diagnosis	Final Diagnosis	Kind of Diagnosis	Icterus Index	Time since onset	Sex	Age	Hemorrhage	Red Blood Cells	Hemo- globin, per Cent	Remarks
41	Pernicious anemia	Hemophilia	Clinical	1.5	1 year	♂	23	++	1,370,000	15	
42	Pernicious anemia?	Leukemia?	Clinical	5.0	0	♂	60	0	1,910,000	15	
43	Pernicious anemia?	Hookworm	Organisms demonstrated	3.0	Pallor	♂		+	2,110,000	75-82	
44	Uncinariasis	Hookworm	Organisms demonstrated	2.0	0	♂	32	0	2,000,000	65	
45	Pernicious anemia?	Splenic anemia	Clinical	3.5	±	♂	45	0	2,602,000	40	Enlargement of liver and spleen, Wassermann reaction ++
46	Sarcoma	Sarcoma thigh	Necropsy Operation	1.8	Pallor	♂		0	1,070,000	65	Pathologic specimen
47	Fibromyomas	Fibromyomas	Clinical	1.0	Pallor	♀	47	Repeated ++	2,100,000	40	Proctoscopy and stool examination, alcoholic 14 years
48	Ulcerative colitis	Ulcerative colitis	Clinical	1.5	Pallor	♂	9	Repeated ++	2,765,000	0	Wassermann repeatedly blood picture not like pernicious anemia
49	Lymphosarcoma of neck	Lymphosarcoma of neck	Necropsy Clinical	2.0	Pallor	♂	74	0	1,400,000	60	Pathologic specimen
50	Pernicious anemia?	Specific anemia	Clinical	2.5	Pallor	♀	40	0	1,580,000	25	Well at present (5/14/25)
51	Hodgkin's disease	Hodgkin's disease	Operation	2.0	Pallor	♀	38	0	2,000,000	40	Blood returned to normal after operation
52	Pernicious anemia	Bleeding fibroid	Operation	3.0	0	♀		Repeated	1,540,000	40	Pathologic specimen
53	Cholelithiasis?	Bleeding gastric ulcer	Operation	3.0	0	♀	40	0	1,540,000	25	Pathologic specimen
54	Carcinoma of sigmoid	Carcinoma sigmoid	Operation	2.5	0	♂	40	Repeated	1,500,000	40	

TABLE 5.—*Various Conditions Exhibiting a High Icterus, Indicated*

Case Number	First Diagnosis	Final Diagnosis	Kind of Diagnosis	Icterus Index	Tau index	Sex	Age	Hemoglobinage	Red Blood Cells per Cent	Hemoglobin, per Cent	Remarks
80	Familial jaundice	Familial jaundice	Clinical	14.0	±	♀	5	0	2,976,000 to 3,530,000	32.5 to 50	Wassermann negative
81	Carcinoma of common duct obstruction	Common duct obstruction	Operation	10.0	++++	♂		0			Mild secondary anemia
82	Carcinoma of pancreas	Chronic cholecystitis and cholangitis	Operation	73.0	+++	♂	35	0	3,232,000	35	
83	Wells disease	Wells disease	Clinical	96.0	++++	♂	55?	0	6,300,000	85	Secondary anemia
84	Polycythemia vera	Polycythemia vera	Clinical	7.0	0	♀	55?	0	4,904,000	65	Pathologic report
85	Cholecystitis	Chronic cholecystitis	Operation	16.0	+	♀	47?	0	Patient had anemia of 3,000,000, followed by drop to 1,000,000 (with two doses of arsphenamin)		
86	Pernicious anemia	Arsphenamin poisoning	Clinical	8.0	0	♂		0			
87	Aplastic anemia	Benzene poisoning	Clinical	8.5	Pallor	♂	30?			23 to 57	
88	Duodenal ulcer	Duodenal ulcer	Operation	8.0	Pallor	♂	49	0	1,800,000	75	Pathologic specimen
89	Typhoid fever	Typhoid fever	Clinical	9.5	0	♀	30?	0	1,490,000	80	Positive Widal and blood culture
90	Acute cholelithiasis	Acute cholelithiasis	Operation	10.5	0	♂	47?	0	3,690,000	60	During attack
91	Acute cholecystitis	Chronic cholecystitis, etc	Operation	39.0	++	♀	46?	0	3,140,000	55	Pathologic specimen
92	Diabetes	Diabetes	Clinical	9.0	0	♀	46	±	1,210,000	70	
93	Acute appendicitis	Acute appendicitis and general peritonitis	Operation	9.5	±	♂		0	1,820,000	85	Associated pericholangitis, Van den Bergh slightly +, bilirubin 50 mg per 100 cc
94	Carcinoma of head of pancreas	Cirrhosis of liver and chronic pancreatitis	Operation	96.0	++++	♂	63	0	4,560,000	85	Wassermann reaction 4+
95	Acute gangrenous appendicitis	General sepsis	Operation	8.0	0	♀	19	0	1,740,000	75	Pyelophlebitis, acute peritonitis, died
96	Acute appendicitis with abscess	Acute appendicitis with abscess and sepsis	Operation	29.0	+	♂	32	0	1,220,000	80	Discharged improved
97	Pernicious anemia	Chronic sepsis and hemolytic jaundice	Necropsy	46.0	++	♂	15	0	610,000	15	Wassermann reaction 3+

normal range, and in cases of carcinoma it was below normal. The interesting point is that not only both series have indexes differing from normal and from one another, but that each range is on the opposite side of normal, making differentiation by means of the icterus index relatively simple.

The fact that hemolytic anemia can be readily differentiated from anemia due to other causes, by means of the icterus index, has been pointed out before by Lepehne⁶ and by Andrewes.⁷

Table 4 presents examples of various conditions in which there was secondary anemia with a low index. Uncomplicated cases of secondary anemia are thus seen to give a uniformly low normal or below normal index. This table also is of special interest for the reason that the cases contained therein were at first wrongly diagnosed clinically as pernicious anemia, and finally turned out to be secondary anemias of one sort or another (Cases 41, 43, 45 and 50).

It is not to be supposed that a diagnosis of pernicious anemia is to be made merely because the patient presents a high index with more or less marked anemia. Thus, Table 5 gives numerous examples of conditions other than pernicious anemia which may give a high index. Many of these conditions can be differentiated with ease clinically and by the history, etc., or with the additional information supplied by Van den Bergh's test.

Likewise, complicating factors must always be borne in mind in the interpretation of any index reading. For example, what would ordinarily be considered a normal or above normal index might be lowered because of hemorrhage, or, on the other hand, the index in a particular case might be raised by such factors as coexisting pneumonia, cardiac insufficiency, or even a slight chronic cholecystitis. Case 19, Table 3, illustrates the point in question. Here we have a case of carcinoma of the sigmoid (proved at necropsy) with severe anemia, showing no metastases in the liver or in the biliary system, but nevertheless giving a high icterus index. The answer lies in the terminal pneumonia which, through hypostatic congestion, caused biliary stasis and gave the raised icterus index. Here, as with almost everything else in medicine, the personal equation must be brought to bear.

SUMMARY

1 The icterus index, rationally interpreted and performed by a simple method, is a distinct clinical aid in the differentiation of the anemias.

(a) Uncomplicated cases of hemolytic anemia have an index above the normal range.

(b) Uncomplicated cases of secondary anemia have a low index, generally below the normal range.

2 Uncomplicated cases of pernicious anemia and the severe anemia simulating it in cases of carcinoma may be readily differentiated by the icterus index, in that the former have an index consistently above the normal (8 to 12.5) and the latter below the normal range

3 (a) The most common factors to be borne in mind which may raise the icterus index above the usual for the primary disease in the subject in question are coexisting pneumonia, cardiac insufficiency and chronic sepsis (especially in the biliary system)

(b) The factor that may lower the icterus index below the usual for the disease in question is hemorrhage, especially a constant, slow (oozing) bleeding, such as occurs in gastro-intestinal new growths

4 The normal range of the icterus index, as determined by the modified Meulengracht method, is from 3.5 to 5.5, with an average of 4.41, for 120 cases

5 It is suggested that the icterus index may be used to follow the course of pernicious anemia and determine, before the blood count falls, when it is advisable to give a transfusion, as well as when the patient is likely to improve spontaneously

THE CONCENTRATION OF THE BLOOD AND OF THE URINE IN DIABETIC TOXEMIA *

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AND

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WITH THE ASSISTANCE OF

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The marked concentration of the blood that is found in the severe diabetic state has been pointed out in a previous article ¹ Here we wish to show the striking changes in the concentration of the urine that are associated with this condition and to indicate the rapid alterations that accompany successful therapy

Urinary nitrogen was determined by the usual Kjeldahl method, sugar by a modification of Benedict and Osterberg's micro method ² in the earlier studies and later by the Shaffer and Hartmann method ³ Urinary phosphate was estimated by the method of Bell and Doisy as modified by Briggs, ⁴ and chlorid by Volhard-Harvey titration The Austin and Van Slyke ⁵ method for plasma chlorid was used in earlier studies, later Van Slyke's ⁶ new method was substituted Plasma phosphate was determined at first by Brigg's ⁴ modification of the Bell and Doisy method and later by the procedure proposed by Benedict and Theis ⁷ The other methods used and the general procedure followed in the examination of the blood have already been described ¹

Treatment during the first few days consisted in giving, in conjunction with insulin, large amounts of fluid and considerable carbohydrate In the most severe cases a diet was not started until after two or three days of treatment Carbohydrate was given in the form of 5 per cent glucose solution subcutaneously and orange juice sweetened with sucrose or lactose by mouth After the first twenty-four hours milk, cream and lactose were often added in sufficient amounts to approximate a replacement diet The 5 per cent glucose solution was given in amounts of 500 c c at a time, together with 1,000 c c of physiologic sodium chlorid solution once or twice in twenty-four hours This we believe

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1 Peters, J P, Bulger, H A, and Eisemann, A J J Clin Invest **1** 435-451, 1925

2 Benedict, S R, and Osterberg, Emil J Biol Chem **48** 51 (Sept) 1921

3 Shaffer, P A, and Hartmann, A F J Biol Chem **45** 365 (Jan) 1921

4 Briggs, A P J Biol Chem **53** 13 (July) 1922

5 Austin, J H, and Van Slyke, D D J Biol Chem **41** 345 (March) 1920

6 Van Slyke, D D J Biol Chem **58** 523 (Dec) 1923

7 Benedict, S R, and Theis, R C J Biol Chem **59** 63 (Aug) 1924

is a valuable aid in increasing the fluid intake, especially in cases with persistent vomiting or coma. Insulin dosage was estimated arbitrarily according to the height of the blood sugar, the persistence of glycosuria and the amount of carbohydrate to be given. It was generally followed within half an hour by a feeding or injection of carbohydrate.

A striking dehydration of the blood has been a prominent feature in all cases of severe diabetic toxemia, and is, we believe, worthy of important consideration in treatment. This dehydration is indicated in the cases shown in Table 1 by the high initial concentration of hemoglobin

TABLE 1—*Summary of Cases*

Case	Day	Cell Volume, per Cent	Hemo- globin, per Cent	Plasma Proteins, per Cent	Carbon Dioxid per Cent by Volume	Blood	
						Sugar, Mg per Cent	Nonprotein Nitrogen Mg per Cent
1	Admission	47.2	22.3	7.71	51.1	742	67
	3	45.1	21.3	6.01	76.5	216	39
	31	40.0	18.4	6.44	54.1	297	22
3	Admission		20.1	8.24	13.4	616	37
	2		20.0	7.66	33.7	344	
	3		16.9	6.14	47.8	349	
	8		16.9	6.44	73.7	249	
5	Admission	45.8	22.5	8.59	16.8	308	37
	2	42.3	21.0	8.28	41.6	142	
	3	40.6	19.0	6.41	49.3	229	
	9	38.1		6.05	60.9	241	
6 First admission	Admission	40.6	18.9	6.95	20.8	300	
	2	36.7	16.7	6.37	38.3	121	
	25	34.2	14.8	6.23	66.2	150	
6 Second admission	Admission	38.2	20.8	7.74	14.5	317	
	1	51.2	19.5	7.60	19.5	429	
	2	49.1	20.3	6.90	43.1	278	
	4	43.8	16.9	6.55	55.6	246	
7	Admission	43.7	20.1	8.19	43.8	234	
	3	40.7	17.6	6.87	55.0	113	
2	Admission	46.2	20.1	7.38	47.8	445	
	2	46.8	22.2	7.67	49.3	274	
4	Admission	48.5	20.0	7.43	7.6	450	46
	2	46.2	20.1	7.40	43.0	174	37
	5	37.7	15.4	6.03	51.7	283	29
8	Admission		19.8	6.39	22.0	341	37
	2		18.3	5.22	50.6	179	24
	10		16.2	4.95	64.7	127	21
	17		14.8	4.98	61.8	155	24
	27		17.5	6.17	67.6		23

and of cells in the blood and of protein in the plasma. These changes appear to be due to a decrease in the water of the blood rather than to an absolute increase in the blood elements affected. The cause of this concentration of the blood is not entirely clear. It does not appear to be related to a diminution in fluid intake. Although vomiting was a prominent feature in some cases, concentration was quite as marked in those cases without vomiting. Considerable dilution was effected generally within twenty-four hours and always at the end of forty-eight hours. In the last column of Table 1 are recorded associated changes in plasma carbon dioxide and blood sugar. It seems obvious that the alterations in blood hydration are not related to the level of the blood sugar. Hyper-

glycemia may persist or even increase as the blood becomes diluted Sherrill and John⁸ have shown that the production of hyperglycemia in normal and in diabetic subjects is often accompanied by blood dilution and oliguria. Such reactions and the changes in blood concentration produced by insulin appear to be quite different from those here described.

Haldane and his associates⁹ have shown that salts that induce acidosis cause concentration of the blood. Fluid retention resulting from administration of sodium bicarbonate is occasionally met with. The edema associated with diabetes is quickly relieved by the administration of acidotic drugs.¹⁰ Without exception, striking blood dilution during the first two days of treatment was associated with an increase in plasma carbon dioxide, but it must be emphasized that anhydremia may be found when there is little or no acidosis. Patient 7, with marked concentration of the blood, showed only a slight decrease in plasma carbon dioxide. Patients 1 and 2 had both received sodium bicarbonate before admission to the hospital, and neither had much reduction in plasma carbon dioxide. The last case is of considerable interest. The patient had a pneumonia and died shortly after the second blood was taken. It was impossible for her to take large amounts of fluid by mouth. In spite of the fact that glycosuria and acetonuria were relieved by insulin the blood became more concentrated. It is interesting that Patient 1 had slight edema of the ankles and shins though there was definite evidence of blood concentration. Between the first two observations of Case 4 there was a considerable increase of carbon dioxide without any associated change of blood concentration.

In Tables 2 to 8 are recorded the results of analysis of each separate specimen of urine voided during the first few days that the patient was in the hospital, together with the associated changes in the blood and plasma. In several instances the study was continued by analyzing the urine from twenty-four hour periods only. Therapy in each case was instituted as soon as the first specimen was obtained. The top row of figures in each table shows, therefore, the state of the blood and urine before treatment was begun. The volume of each specimen of urine is recorded in the third column. Estimations of the rate of urine excretion, shown in Column 3, were obtained by dividing the volume by the

8 Sherrill, J. W., and John, H. J. *J. Metabolic Res.* **1** 109 (Jan.) 1922.

9 Baird, M. M., Douglas, C. G., Haldane, J. B. S., and Priestly, J. G. *J. Physiol.* **57** 41 (Jan.) 1923. Haldane, J. B. S., Hill, R., and Luck, J. M. *J. Physiol.* **57** 301 (June) 1923.

10 Falta, W. *Wien Arch. f. inn. Med.* **5** 581 (Jan.) 1923. Atchley, D. W., Loeb, R. F., and Benedict, E. M. *Physicochemical Studies of Calcium Chlorid Diuresis*, abstr., *J. A. M. A.* **80** 1643 (June 2) 1923. Rockwood, Reed, and Barrier, C. W. *Calcium Treatment for Edema*, *Arch. Int. Med.* **33** 643 (May) 1924. Keith, N. M., Barrier, C. W., and Wheland, Mary. *Treatment of Nephritis and Edema with Calcium*, *J. A. M. A.* **83** 666 (Aug. 30) 1924.

TABLE 3—Case 3, Third Admission

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(1)	(2)	(3)	(4)	(5)	(6)			(7)		(8)	(10)	(11)	(12)			(13)		(14)		(15)	(16)	(18)		(19)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
					Volume, Hour, C c	Gm per Liter	Gm per Hour	Gm per 24 Hours	Sodium Chlorid, Gm per Liter				Glucose, Gm per Liter	Ice tone	Protein, per Cent	Carbon Dioxid, per Cent by Volume	Sodium Chlorid, Mg per Cent	Inorganic Phos, Mg per Cent	Oxygen Capacity, per Cent by Volume			Nitrogen, Mg per Cent	Sugar, Mg per Cent																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						

time intervening between that and the preceding voiding. Of course there was no assurance that the bladder was emptied completely each time and the irregularities encountered were to be expected. The nitrogen excreted each hour, shown in Column 6, was estimated in a similar way. Column 7 shows the nitrogen excreted during twenty-four hour periods, starting at the time of admission. One specimen frequently overlapped two twenty-four hour periods. The nitrogen allotted to each was then estimated by interpolation. Columns 8, 9 and 10 record the concentrations of sodium chlorid, inorganic phosphorus and glucose, respectively, in grams per liter, Column 10 the relative intensity of Legal's nitroprussid test for acetone.

In all cases of severe diabetic toxemia the dehydration of the blood was associated with the production of urine containing remarkably low concentrations of nitrogen and sodium chlorid. As the condition of the patients improved and the blood became diluted the concentration of these substances in the urine increased with surprising rapidity. These paradoxical changes occurred in the absence of any constant variation in the rate of urine excretion. Furthermore, there seemed to be no relationship between urine concentration and any individual constituent of the blood. The nitrogen and salt intake was invariably low and could not possibly have accounted for these changes.

In each case of severe diabetic toxemia the concentration of nitrogen in the admission specimen of urine was low. In four instances concentrations as low as 2.5, 2.6, 2.7 and 2.8 gm. per liter, respectively, were encountered. The rate of urine excretion could not be determined in these cases before starting treatment, but the volume output immediately afterward suggests that it was not excessive and probably was relatively low. Apparently oliguria and even anuria may frequently occur in diabetic toxemia. An increase in protein metabolism in this condition is well known and it seems rational to conclude that we are concerned here with a definite failure to eliminate nitrogen. Table 2 shows the study of a case of severe diabetic toxemia. The concentration of nitrogen in the urine (Column 5) was very low on admission but increased steadily and became comparatively normal in less than eighteen hours. The rapid increase in the rate of excretion (Column 6) is even more striking. At a later admission the same patient presented similar phenomena (Table 3). In this one instance the nonprotein nitrogen of the blood and the rate of nitrogen elimination increased simultaneously (Column 18).

In the study of Case 5 (Table 4) the concentration of nitrogen in the urine rose from 6 to 16 gm. per liter in twenty-four hours. Changes of a similar nature are also recorded in Tables 5, 6 and 7. In all of these cases, following the institution of treatment, there was a steady

and rapid increase in the nitrogen concentration of the urine until normal or relatively high values were obtained. Almost without exception there was also an increase in the rate of urine excretion during this period resulting in a striking increase in the rate of nitrogen elimination. In some cases the initial rise in the rate of elimination reached an abnormally high point, later declining to a more or less constant level. This is well illustrated in Tables 2, 4 and 6. The maximum rise occurred at the end of about thirty-six hours although normal concentrations were frequently present at the end of eighteen hours. The excessive nitrogen excretion that was frequently found during the second and third days suggests that during the period of anhydremia and dilute urine there is a retention of nitrogen that is swept out as the dehydration is overcome. Several of these cases have not shown much retention of nitrogen in the blood, but in Case 1 (Table 1) on admission there was a definite elevation of nonprotein nitrogen that fell to normal in twenty-four hours. In two other instances, Cases 4 and 8, the blood nonprotein nitrogen diminished as the concentration and elimination of nitrogen in the urine rose.

The alterations of the urinary sodium chlorid concentration were similar to, but more marked than, the nitrogen changes. The dehydration was attended by extremely low concentrations of sodium chlorid in the urine (Column 8 in each table). The admission urine from Patient 3 (Table 3) was practically chlorid-free. With adequate treatment the urinary chlorid concentration rose to many times the original value in less than twenty-four hours. The steady increase and maximum rise corresponded in time to the similar changes of nitrogen. With Case 3 (Table 2) the plasma chlorid decreased somewhat, yet during the same period the chlorid in the urine increased from 0.4 to 6.2 gm per liter. In only one instance (Table 4) was the increase of chlorid in the urine associated with a great increase of plasma chlorid.

Similar paradoxical changes were encountered in the relationship of blood and urine sugar. This is illustrated by Case 3 (Table 2). The urine at the time of admission contained only 7.6 gm of glucose per liter while the blood sugar was 616 mg. About twelve hours later the urine sugar had increased to 21.7 gm per liter while the blood sugar had fallen to 344 mg. Although the blood sugar decreased about one half, the urine sugar increased approximately threefold. Similar but less marked changes in blood and urine sugar were found in Case 6 (Table 6). It is apparently a well known fact that with the approach of diabetic coma the sugar in the urine may disappear.¹¹ Joslin¹² states,

¹¹ Epstein, A. A., and Felsen, J. *Am J M Sc* **153** 58 (Jan) 1917.

¹² Joslin, E. P. *The Treatment of Diabetes Mellitus*, New York, 1923, p. 380.

"It is certainly true that one often encounters falling percentages of urinary sugar as coma advances" Epstein, Reiss and Branower¹³ have shown that high blood sugar frequently occurs after operation without glycosuria and that at the same time there is a diminution in phenol-sulphonephthalein excretion

The phosphate of the urine in three cases was studied during the initial period of rapid improvement and the observations of other authors confirmed. Quite unlike the nitrogen and chloride, the urinary phosphate concentration on admission was relatively high and fell rapidly with treatment. In each case this decline was followed by a rise reaching concentrations in two instances much greater than those found in the initial urine specimens. The reduction may be ascribed to the rapid removal of phosphate from the blood in the demobilization of carbohydrate. For the later rise no obvious explanation offers itself.

Fitz¹⁴ has reported studies of the renal function in seven cases of impending or true diabetic coma. High blood urea was frequently found and in all cases the concentration of urea and sugar in the urine was low in comparison with that of the blood. In one fatal case in which the acetone bodies of the blood and of the urine were studied urinary acetone decreased steadily without any corresponding diminution of blood acetone. Fitz suggests that the acetone bodies may exert a specific toxic effect on the kidneys. He made no determinations that would indicate the changes in the concentration of the blood.

Warburg¹⁵ has reported some cases of "diabetic coma with uremia". Two patients were admitted to the hospital in coma with very high blood sugar and Kussmaul breathing. At first diuresis was slight and very high urea values were found in the blood (129 and 270 mg per cent). In one case there was no albumin in the urine and in the other a moderate amount. Both patients were restored to health with insulin therapy, the blood urea falling to normal in the course of about two weeks. Later kidney function tests showed no definite abnormalities. He describes another case of a girl, aged 20, with known diabetes, who was admitted in coma. She was treated inadequately with insulin and fluids, and developed anuria and died. No blood urea determinations were made. The kidneys at necropsy were said to present gross evidence of acute parenchymatous nephritis, but no histologic study was made. Warburg remarks that "it does not appear to be generally acknowledged that the kidneys often become strongly deficient in diabetic coma". He has reviewed the history of diabetic coma thoroughly and states that oliguria and anuria are common.

¹³ Epstein, A. A., Reiss, Joseph, and Branower, Jacob. *J. Biol. Chem.* **26** 25 (Aug.) 1916.

¹⁴ Fitz, Reginald. *Kidney Function in Diabetes*, *Arch. Int. Med.* **20** 809 (Nov.) 1917.

¹⁵ Warburg, Erik. *Acta med. Scandinav.* **59** 301, 1925.

TABLE 7—(cont.)

[illegible]

TABLE 8—Case 8

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	Plasma			Blood		
			Volume, C c	Volume, per Hou., C c	Nitrogen		Sodium Chlorid, Gm per Liter	Inor- gmic phos- phorus, Gm per Liter	Glu- cose	Ac- tone	Pro- tein, per Cent	Carbon Dioxid, per Volume Cent	Sodium Chlorid, Mg per Cent	Inorganic Phos- phorus, Mg per Cent	Oxygen Capac- ity, per Volume Cent	Non protein Nitrogen, Mg per Cent	Sug- ar Mg per Cent
Date, 1925	Time	Volume, C c	Volume, per Hou., C c	Gm per Liter	Gm per Hou.	Gm per 24 Hours											
March 4	6 00 p m	385	263	3.24	0.740		1.50	0.195	4+	2+	6.39	22.0					
March 5	11 00 p m	1 315	227	2.81	0.366	1 17	2.72	0.138	2+	3+							
	5 10 i m	1,400	150	2.49	0.305		2.12	0.158	2+	+							
	11 30 a m	950	263	2.03	0.483		2.54	0.112	3+	2+							
	4 40 p m	1,300	263	1.72	0.370		2.00	0.072	2+	+							
March 6	10 35 p m	1,370	228	1.77	0.359		1.40	0.053	0	0							
	3 45 i m	830	160	1.41	0.226		1.26	0.090	+	+							
	8 30 a m	1,050	217	1.41	0.305		1.36	0.127	+	2+	5.22	50.6		1.8	18.3	24	179
March 6-7	24 hours	4,375	(190)	1.34		6 14	1.49										
March 8-9	24 hours	2,315	(96)	1.95		4 52	1.34										
March 9-10	24 hours	2,335	(97)	1.06		2 48	1.75										
March 11	8 30 a m										1.95	64.7	0.22	3.3	16.2	21	127

It is impossible to draw any definite conclusions as to whether there is a causal relationship between the concentration of the blood and this inability to concentrate urine. It is interesting to consider the possibility of an increase of osmotic resistance due to an increase in the plasma colloids. The plasma proteins in some of our cases were not extremely high. In all of the cases reported here there were more or less albumin and hyaline and granular casts in the urine before treatment was begun. The albumin and casts in these toxic states suggest local kidney changes. Some disturbance of renal circulation might well explain the functional alterations observed, structural damage to the kidney is hardly conceivable in view of the rapid recovery of function that follows well directed therapy. The excessive concentration of the urine encountered during the second or third days without constant comparable variations in blood constituents suggests that alterations in general tissue metabolism may play some rôle in the phenomena observed.

In this connection it is interesting to contrast Cases 8 and 4. Patient 8 was admitted to the hospital after a long period of malnutrition. He complained chiefly of weakness and marked loss of weight. There had been no recent change in his condition and acidosis had probably been present for some time. His diabetes was moderately severe but his condition was not immediately serious. In this case the dilution of the blood following treatment was quite characteristic but the concentration of nitrogen in the urine decreased steadily, quite in contrast with the other cases reported here. It is also to be noted that this patient had a marked diuresis from the first. The concentration of chlorid during the first twenty-four hours of treatment was somewhat greater than that of the admission urine, but the change was not great. An insufficient diet rather high in proteins probably explains the high nitrogen excretion on admission. The diminishing excretion following treatment was probably due to the fact that increased utilization of carbohydrate and fat permitted the storage of protein to compensate for the preceding period of undernutrition.

Patient 4 also had diabetes of moderate severity but was admitted to the hospital in a very serious condition. He had been careless in following treatment and the day before admission became cold, vomited several times and later developed headache and severe epigastric pains. On admission he was in a state of restless coma. Hyperventilation was extreme. His skin was cold and cyanotic, the temperature subnormal and the pulse weak and rapid. He was obviously very sick. To this condition as contrasted with that of Case 8, we have applied the term "toxemia." The term has not been used as synonymous with either acidosis or ketosis but to distinguish the cases in which the condition

appears immediately grave, cases generally complicated by infection and showing in addition to marked ketosis and dehydration, stupor or coma and various manifestations of circulatory failure. With improvement in his condition this patient did exhibit the characteristic rapid alteration in the concentration of the urine, but a blood examination forty hours after the institution of treatment revealed no change in concentration, although the patient had been given large amounts of fluids and the plasma carbon dioxide had increased 35 per cent by volume.

There was therefore quite a contrast between the conditions of the two patients, conditions effecting a marked difference in the production of urine. The former showed a rapid dilution of the blood but not the changes in the concentration of the urine which have been characteristic of diabetic toxemia. The latter showed the increasing concentrations of the urine while the concentration of the blood remained unaltered. This suggests that the dilute urine may not be directly dependent on the dehydration of the blood but on some other changes characteristic of the toxemia.

These studies have emphasized certain important considerations in treatment. They not only indicate clearly the importance of giving large amounts of fluids but also the urgent need of combating the toxic destruction of protein. These considerations are all the more important in cases complicated by infection in which there is additional protein destruction, and in cases complicated with kidney disease in which there is a greater tendency for nitrogen retention. It seems clear that glycosuria in itself can be disregarded and large amounts of carbohydrate given in conjunction with insulin, not only to reduce ketosis and acidosis but as a means of sparing the toxic destruction of proteins.

CONCLUSIONS

In severe diabetic toxemia with marked dehydration of the blood the concentration of nitrogen and chlorid in the urine is extremely low. With adequate treatment there is a rapid dilution of the blood and a rapid increase in the concentration of these substances in the urine.

There is no direct relationship between the alterations in the blood and the urine and none of the changes appear to be significantly affected by the presence or degree of hyperglycemia or glycosuria.

The bearing of these phenomena on the treatment of diabetic toxemia has been considered.

REPORT OF CASES

CASE I—A woman, aged 47, who was known to have had diabetes for at least a year, entered the hospital with the history of cough and "a cold in her chest" for two weeks and persistent vomiting for four days. There had been nocturia for three or four years and pyuria at intervals. For several days before admis-

sion she had received large doses of sodium bicarbonate. She was well nourished but appeared acutely ill. Respirations were not increased in rate or depth but she was definitely drowsy. The mouth was dry. There was slight cardiac enlargement and scattered râles throughout the lungs. There was slight subcutaneous edema of both shins and ankles. The temperature was 96 F, and the pulse 100 per minute. The systolic blood pressure was 180, and the diastolic 98. The urine showed considerable sugar and acetone and an abundance of pus. The blood nonprotein nitrogen was 67 mg per hundred cubic centimeters.

The patient entered the hospital in the afternoon of December 11. By the following morning she had received 30 units of insulin but vomiting continued, interfering with the administration of fluid. The blood nonprotein nitrogen was still 67 mg. During the second day the râles in her chest cleared and she seemed to be retaining some fluid. At the time of the second blood examination, about forty hours after admission, she had received 64 units of insulin and was somewhat improved. The edema was slightly more marked. The urine contained no sugar or acetone. Because of the continuous vomiting, the low plasma chlorid and gastric anacidity, she was given 1,000 cc of physiologic sodium chlorid solution by hypodermoclysis. Improvement was rapid. Five days after admission the phenolsulphonephthalein excretion was 63 per cent in two hours and ten minutes. She was finally discharged on a maintenance diet without insulin.

CASE 2—The patient was a woman, aged 57, who had had glycosuria and hypertension for at least three years. Five weeks before admission she developed a cold which gradually grew worse. Two days before admission there was marked respiratory distress with blood tinged sputum. She had been given sodium bicarbonate by mouth. On admission the patient was semicomatose, with rapid shallow respirations and marked cyanosis, obviously she was extremely ill. The temperature was 101 F, the pulse 120 and respirations 42 per minute. The lungs showed evidence of extensive generalized bronchopneumonia. The heart was slightly enlarged and the blood pressure elevated, the systolic being 170 and the diastolic 80. The urine showed considerable sugar, a moderate amount of acetone, a faint trace of albumin and an occasional hyaline cast.

She was admitted at 7 p. m., Dec. 13, 1922. It was very difficult to give anything by mouth, and very little nourishment was administered. By means of small doses of insulin the urine was freed from sugar and acetone by the following morning. Later that day the patient vomited several times, a total of 600 cc. She became involuntary of urine and feces, grew progressively worse and died after being in the hospital fifty-four hours. The total fluid intake while in the hospital was about 4,000 cc.

CASE 3—A woman, aged 50, entered the hospital, July 26, 1923, complaining of weakness, thirst and polyuria. The symptoms had begun suddenly about eight weeks earlier. She was slightly emaciated but not extremely ill. There was no hypertension. The urine was negative except for sugar. After three weeks she was discharged on a maintenance diet requiring small doses of insulin.

After discharge from the hospital the improvement in her general condition was not marked. The few weeks before the second admission cooperation and consequently treatment were unsatisfactory. Four days before the second admission she was seized with severe abdominal pains and vomiting. The pain and vomiting persisted. She was moderately emaciated, with dry skin and mucous membranes, breathing deeply with evident air hunger. She was nearly comatose and obviously quite ill. The pulse was rapid but regular. There was some tenderness in the epigastrium but no spasm or rigidity. The weight was 125 pounds (56.7 kg.). The urine showed albumin, a few hyaline and granular casts and large amounts of sugar and acetone. The blood count showed 6,100,000 red blood cells and 13,200 leukocytes. She was admitted at 10 p. m., Nov. 19, 1923. During the first twenty-four hours she received 185

units of insulin, together with fluids containing sucrose, by mouth and 3,100 c c of physiologic sodium chlorid solution and 700 c c of 5 per cent glucose by hypodermolysis. At the end of this period there was very little change in her general condition. During the second twenty-four hours she received 85 units of insulin, with continuation of the sucrose by mouth. On the morning of the third day she was definitely improved and her diet was started. She was finally discharged on her previous diet requiring larger doses of insulin.

She entered the hospital a third time, Sept 6, 1924, with a history similar to that preceding the previous admission except that vomiting and abdominal pain had continued for only twenty-four hours. She was very weak and drowsy with deep respirations and evidence of marked dehydration. The heart was rapid with numerous extrasystoles. The urine was negative except for sugar and acetone. She was admitted about 10 30 p m and at once given 1,000 c c of physiologic sodium chlorid solution and 500 c c of 5 per cent glucose by hypodermoclysis with 40 units of insulin. Orange juice with sucrose was given in small amounts at a time. During the night she received 40 units more of insulin and the following morning nausea and abdominal pain had lessened. During the day, however, vomiting continued, and she was again given physiologic sodium chlorid solution and 500 c c of 5 per cent glucose under the skin and fluids limited to 50 c c every half hour by mouth. By midnight she had received an additional 95 units of insulin and her condition was much improved. She continued to improve clinically. On the morning of September 10 she got out of bed without permission, became dizzy and vomited. Vomiting continued and there was a return of acidosis and air hunger. The plasma carbon dioxide fell to 28 per cent by volume. The diet was stopped and she was allowed only orange juice and water by mouth, with insulin as required. Under these circumstances she recovered rapidly and the diet was resumed the next day. After one month in the hospital she was discharged on a diet containing 50 gm of protein, 150 gm of fat and 100 gm of carbohydrate, requiring 136 units of insulin daily.

CASE 4—A man, aged 33, was first admitted to the hospital in November, 1923, for the repair of bilateral inguinal hernia. He did not regain his strength after the operation and later developed marked polyuria and began to lose weight and strength. Glycosuria was diagnosed and he again entered the hospital in March, 1924. At this time he was fairly well nourished and had no acidosis. The weight the day of admission was 145 pounds (65.8 kg). The urine sugar cleared rapidly with insulin and he was discharged on a diet containing 70 gm of protein, 200 gm of fat and 100 gm of carbohydrate, requiring 10 units of insulin daily.

The patient apparently did quite well on this regimen, but about two months before the second admission stopped insulin and dieting. Polydipsia and polyuria then became marked and he began to lose weight. A productive cough developed. The day before admission he became very cold, vomited several times and later developed headache and severe epigastric pain. He could not remember anything that happened the day of admission.

He was admitted, March 22, 1925, at 4 40 p m obviously in a very serious condition. He was comatose but restless and thrashed about the bed, he was considerably emaciated. The respirations were very deep and increased in rate. The skin was quite cold with slight general cyanosis, the mouth was extremely dry and the throat was inflamed. The heart was regular and not enlarged the rate being 84. The lungs showed dulness, bronchial breathing and rales in the right interscapular region. The abdomen was negative, the liver not palpable. The systolic blood pressure was 80, the diastolic 60, the temperature was 96 F. The urine showed considerable sugar and acetone, a moderate amount of albumin and many granular casts. The sputum showed acid-fast bacilli. The weight eight days after admission, was 133 pounds (60.3 kg).

Soon after admission he was given 1,500 c c of physiologic sodium chlorid solution subcutaneously. When urged he would take fluids by mouth and was given, in addition to water, sweetened orange juice and, later, mixtures of milk, lactose and cream. On the second day he also was given 1,000 c c of physiologic sodium chlorid solution and 500 c c of 5 per cent glucose. By the time of the second blood examination, forty hours later, he had received approximately 285 gm of carbohydrate, 40 gm of fat and 15 gm of protein, together with 8,600 c c of fluid and 210 units of insulin. Improvement had been marked. Four weeks later he was on a diet containing 75 gm of protein, 250 gm of fat and 125 gm of carbohydrate, requiring 15 units of insulin daily.

CASE 5—A woman, aged 56, was brought to the hospital in coma. Five years before she had had a similar illness, said to have been diabetic coma. Following this she lived on a restricted diet until a few months before admission. During those few months the urine showed varying amounts of sugar but the patient felt well, losing only slightly in weight. Two days before she entered the hospital she became stuporous and up to the time of admission had not been aroused from this condition.

She was completely unconscious. The skin and mucous membranes were quite dry. The pulse was 108 per minute and regular. The respirations were deep. The temperature was 101 F. The heart was moderately enlarged and the peripheral arteries showed definite sclerosis. There were no hypertension and no signs of cardiac failure. Over the base of the right lung there was dulness with diminished breath sounds and râles. Examination of the abdomen revealed nothing of special note. There were no neurologic signs. The urine showed a slight trace of albumin and considerable sugar and acetone. The sediment contained numerous hyaline casts. The blood count showed 6,250,000 red blood cells and 27,000 leukocytes.

The patient was admitted to the hospital at 11 a m, Jan 28, 1924. With constant urging she could be made to swallow water and sweetened orange juice. In addition 2,000 c c of physiologic sodium chlorid solution and 1,000 c c of 5 per cent glucose were given by hypodermoclysis during the first day, making a total of 5,000 c c of fluid. By the time of the second blood examination the following morning, she had received 200 units of insulin. The mental condition was unchanged, but the respirations had quieted down. During the next twenty-four hours she received, in addition to fluids by mouth, physiologic sodium chlorid solution and 500 c c of 5 per cent glucose by hypodermoclysis, together with 145 units of insulin. On the third day she was apparently conscious and responded to stimuli but did not speak and seemed somewhat confused. With further treatment her general condition improved, but aphasia persisted. Three weeks later she developed a pneumonia and at about the same time a pyelitis. These conditions cleared up slowly. She was discharged from the hospital four months later on a diet containing 50 gm of protein, 150 gm of fat and 100 gm of carbohydrate and requiring 45 units of insulin daily. Later insulin was gradually discontinued. The aphasia has persisted to the present time.

CASE 6—A white man, aged 36, whose diabetic symptoms started suddenly ten weeks before admission, following an attack of severe acute epigastric pain, had lost considerable weight and complained of great weakness. The week before admission his general condition was definitely worse, the appetite had failed, marked constipation had developed, and weakness had become extreme. He had been nauseated and just before admission vomited once. He looked quite sick. Except for marked emaciation and definite hyperpnea, physical examination showed nothing important. His mouth was not particularly dry and there was no noticeable stupor. His weight was 103 pounds (46.7 kg). The systolic blood pressure was 124, the diastolic 86. The admission specimen of urine showed a slight trace of albumin, considerable sugar and acetone and

a negative sediment The blood showed 5,500,000 red blood cells and 11,200 leukocytes The blood plasma before treatment revealed a moderate lipemia which had cleared by the next examination

He was admitted to the hospital on the evening of Feb 8, 1924, and received nothing but non-nutrient fluids over night The first blood and urine were obtained the next morning before treatment was started He was put on a diet at once During the first five days part of the carbohydrate was given as orange juice February 9, he was given 50 gm of protein, 150 gm of fat and 110 gm of carbohydrate with 90 units of insulin The fluid intake was 3,400 cc On the following morning there was evidence of definite improvement February 10, he received 50 gm of protein, 150 gm of fat and 145 gm of carbohydrate with 35 units of insulin The fluid intake was 3,500 cc By the end of this period there had been considerable improvement He was discharged at the end of one month aglycosuric, on a diet of 50 gm of protein, 200 gm of fat and 100 gm of carbohydrate, without insulin

Following discharge from the hospital the patient did fairly well After a few days insulin had to be resumed At first from 10 to 20 units each week was sufficient but after several months his requirement had gradually increased to 40 units daily The diet had remained constant except for occasional indiscretions Later it appears that he became somewhat careless about his diet and four days before the second admission stopped taking insulin The night before admission there was a sudden onset of nausea and vomiting followed by intense abdominal pain Vomiting continued up to the time of admission He was emaciated and greatly dehydrated Respirations were almost maximum in depth He was somewhat stuporous and not clear mentally The mucous membranes were slightly cyanotic The pulse was rapid and thready The systolic blood pressure was 112, the diastolic 85, the temperature was 99 F The abdomen was held tense but nowhere showed definite spasm The admission specimen of urine contained considerable sugar and acetone, a large amount of albumin and many coarse and fine granular casts Examination of the blood showed 5,600,000 red blood cells and 30,000 leukocytes The condition seemed very grave and the abdominal symptoms were so marked that surgical operation was seriously considered

He was admitted to the hospital the second time at 1 45 p m, November 10 Soon after admission he was given 1,000 cc of physiologic sodium chlorid solution and 500 cc of 5 per cent glucose by hypodermoclysis, with 40 units of insulin Four hours later the abdominal pain had subsided and vomiting stopped For the next four hours he was given 50 cc of orange juice every half hour with a total of 60 units more of insulin From then until 5 p m, November 11, he received 25 cc of orange juice, 25 cc of water and 25 gm of lactose every half hour with an additional 160 units of insulin Twelve hours after admission he showed considerable improvement, hyperpnea had practically disappeared November 12, a diet containing 50 gm of protein, 150 gm of fat and 100 gm of carbohydrate was started He was discharged from the hospital on the twenty-fifth day requiring 60 units of insulin daily

CASE 7—A Jew, aged 37, entered the hospital with a history of polyuria, polydypsia, weakness and loss of weight during the previous three years There had been a recent marked exacerbation of symptoms with a cold and double otitis media He was of unusually small stature, extremely emaciated and markedly dehydrated On admission both ear drums had ruptured and there was a marked rhinitis The respirations seemed somewhat increased but the plasma carbon dioxide was normal The urine contained a trace of albumin and a large amount of sugar and acetone, microscopic examination was negative A diet containing 50 gm of protein, 100 gm of fat and 50 gm of carbohydrate was started at once Improvement was rapid During the two days between the first and the second blood examinations, he received 150 units of insulin and

(though not advised by the attending staff) 40 gm of sodium bicarbonate. He was discharged later on a diet containing 50 gm of protein, 175 gm of fat and 100 gm of carbohydrate with 45 units of insulin daily.

CASE 8—A man, aged 28, was admitted to the hospital after about four months of diabetic symptoms. These symptoms had developed suddenly about three weeks after an acute upper respiratory tract infection. Polyuria and polydypsia were marked, and he had lost considerable weight and strength. There had been no particular change in the general condition during the few weeks before admission. There was a marked family history of obesity but the patient was a very small man, his average weight being from 110 to 115 pounds (49.9 to 52.1 kg). On admission he was extremely emaciated, weighing only 65 pounds (29.5 kg). He was mentally clear and not drowsy. There was no cyanosis. The mouth was somewhat dry. The respirations were slightly but definitely increased in depth. The lungs were clear and the heart negative. The liver edge was palpable 1 inch (2.5 cm) below the costal margin. The urine showed a very slight trace of albumin, considerable sugar and acetone and nothing important in the sediment.

He started at once to take a diet containing 50 gm of protein, 150 gm of fat and 100 gm of carbohydrate. During the first twenty-four hours he received in addition 600 cc of orange juice and 130 units of insulin. Rapid improvement followed and he was discharged four weeks later on a diet containing 60 gm of protein, 175 gm of fat and 110 gm of carbohydrate, requiring 25 units of insulin daily.

THE DIAMETER OF THE RED BLOOD CELLS IN THE DIFFERENTIATION OF ANEMIAS

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Advances in the treatment of Addison's anemia in the last few years are so full of promise that it is now of more importance than ever to make an early and decided diagnosis of this disease. If the full benefits of the newer treatment¹ are to be obtained, the diagnosis should be made at least early in the first period of anemia. Indeed, in some cases it is possible to make the diagnosis so early, even before the anemia is apparent, that it may not be in vain to hope that at times the disease as we now know it may be entirely prevented.

It is to aid in the more exact differentiation of the anemias that this work is presented, for it is believed that careful measurement of the red blood cells helps materially in such differentiation. This thesis was presented originally by Price-Jones in England,² was later enlarged on by Shackle and Hampson,³ and more recently by Hurst⁴ and his co-workers. To the present time, however, little has appeared in the American literature on the subject, and no "red cell diameter curves" have been published. Capps⁵ in 1903 measured the diameter in a number of diseases, but his notes on technic are incomplete, and he drew up none of the curves later devised by Price-Jones.

In this paper is described a simplified technic for the measurement of red cells and for the construction of the curves, curves are shown from normal subjects, from patients with Addison's anemia, from patients with secondary anemia, and from patients with a few other diseases, and the conclusions that one is forced to adopt from this work are indicated.

The ingenious method for recording the diameters of red cells devised by Price-Jones is shown by the curve in Figure 1. The solid line is a reconstruction much reduced of the normal curve recorded by Price-Jones on the measurement of 10,000 red cells in twenty nor-

1 Hurst, A. F. Medical Essays and Addresses, New York, Paul B. Hoeber, 1924. Vanderhoof, D. The Etiologic Relation of Achylia Gastrica to Combined Sclerosis of the Spinal Cord, Arch. Int. Med. **32** 958 (Dec.) 1923.

2 Price-Jones, C. The Diameter of Red Cells in Pernicious Anemia and in Anemia Following Hemorrhage, J. Path. & Bacteriol. **25** 487 (Oct.) 1922.

3 Shackle, J. W., and Hampson, A. C. Megalocytic and Nonmegalocytic Anemia, Giv's Hosp. Rep. **74** 193 (April) 1924.

4 Hurst (Footnote 1).

5 Capps, I. A. A Study of Volume Index, J. M. Res. **10** 367, 1903-1904.

mal subjects. The ordinates show the diameter of the cells in microns; the abscissa, the number of cells of each different diameter. At a glance one can see the largest and the smallest cells encountered in a spread (i. e., the degree of anisocytosis), the frequency with which a particular size occurs, and the position of the peak of the curve, which corresponds rather accurately with the average diameter.

TECHNIC

The technic for measuring the diameter of the cells as described by Price-Jones and as modified by Shackle and Hampson is exceedingly difficult to accomplish. It consists essentially of projecting the microscopic field either by means of a camera lucida or a projecting microscope to a screen, on which the image of the cell is either measured directly or its outline drawn and the outline measured. Two diameters are measured on each cell, the average taken, and a curve drawn from the average diameters of 500 cells. Such a procedure is so laborious and time consuming that it is hardly practical for use in clinical medicine.

The procedure used in this work was devised before we knew the technic of the English workers. Our measurements were made by means of a Leitz ocular micrometer. This is a glass disk, on which is engraved an arbitrary scale of fifty divisions, which fits into the ocular of the microscope so that the image of the scale focuses on the image of the blood film on the microscope stage. The scale is first calibrated for the particular set of lenses and tube length to be used, by means of an object micrometer, a slide on which are engraved lines a known distance apart.

When the value in microns of the divisions of the scale of the ocular micrometer is known, one is ready to proceed with the measurements. A blood film is brought into focus. Two or three cells lying on the scale are measured to fifths of a space. The slide is moved a short distance by the mechanical stage, and the two or three cells that happen to lie on it again are measured. As the measurements are made they are called off and an assistant marks off a space for each cell in the proper column on cross-ruled paper. In this way a curve is built up as the count proceeds. When 200 cells are measured the final curve is drawn by connecting the heads of the columns, and then a scale is drawn in showing the value of each column in microns. To complete the curve the numerical average diameter of the cells is determined.

The foregoing procedure may be criticized in a number of respects. We have no doubt sacrificed accuracy for practicability to some extent, but our results agree so closely with the results of the English workers

that we feel that this procedure, by which a red cell diameter curve can be made with about the same expenditure of time and labor as a differential white count, warrants consideration

It will be noted that we measure only one diameter in each cell instead of averaging two. This necessitates selecting round cells for measurement, for it is obvious that oval cells would give incorrect values. No doubt selecting just the round cells causes certain inaccuracy, but the error probably works in both directions and tends to neutralize itself.

Even averaging two diameters does not, as Capps points out, give a perfect picture of the size of the red cells, which are, of course, three dimensional bodies. The thickness of the disks no doubt varies and may be of some clinical significance. Capps feels that the volume of

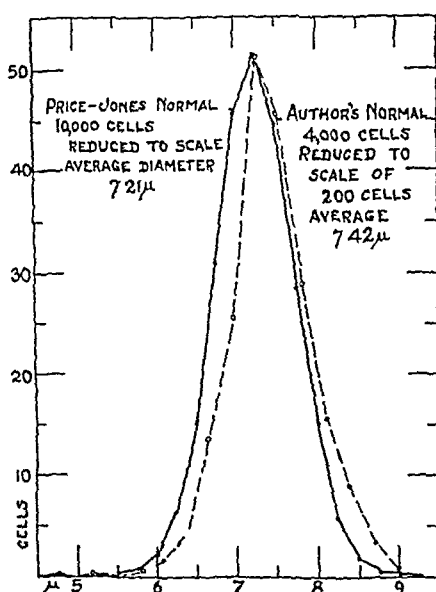


Fig 1—Author's normal red cell diameter curve compared with that of Price-Jones

the red blood cells can be measured accurately by his method of estimating the "index volume." In the strict sense our curves are not volume curves but diameter curves. This does not, however, invalidate the conclusions that may be drawn from the red cell curves or their significance in differential diagnosis.

Our method of sampling is possibly less accurate than if we measured all the cells in a given field. One could, of course, make the curves entirely inaccurate if one measured only the large cells or only the small cells, but successive counts on the same slide give curves that are so similar that we feel a reasonably accurate sample is obtained if one moves quickly across a slide and counts all of those round cells that happen to fall on the micrometer scale.

Two hundred cells do not, of course, give as representative a sample as the 500 that the English workers use. Price-Jones⁶ has estimated that according to statistical laws an error of 0.2 microns is to be expected if 500 cells are counted, 0.32 microns if 200 cells are counted. In practice our variations have not been this much. In measuring two sets of 200 cells each on the same slide our mean diameters have not been more than 0.3 microns apart, a difference that we feel is not of enough importance to warrant measuring more cells. Measuring 500 cells is a difficult task even with the simplest technique. It cannot be accomplished at one sitting and tends to make the procedure impracticable for clinical use. Two hundred cells can, however, be measured in from ten to fifteen minutes after a little experience.

The best argument for the foregoing procedure is the fact that our curves agree so closely with those of the English workers. In Figure 1 and all succeeding figures, the broken line represents our normal curve obtained from the measurement of about 4,000 cells in nineteen normal subjects, reduced to 200 cells. It will be noted that the contour of the curve corresponds quite closely with that of Price-Jones. The two limbs of our curve, however, are to the right of the limbs of the Price-Jones curve, which makes our average normal diameter 7.42 microns instead of 7.21 microns.

This brings up one of the most important and difficult parts of the procedure no matter what technique is used, i. e., calibrating the micrometer scale. Whether one measures the cells directly by means of an ocular micrometer or whether one measures the diameter of the projected image by means of a millimeter rule, one must first calibrate the scale used with an object micrometer in order to determine the value of the divisions of the scale in microns. One cannot depend on the theoretical magnification of a certain set of lenses at a certain tube length and reduce the reading by this magnification.

We calibrated our micrometer with a Zeiss Objektmikrometer on which are engraved lines 0.01 mm apart, with two counting chambers on which the double ruled lines are 25 microns apart, and with a slide engraved by Queen and Company in 0.001 inch. These slides gave slightly different readings. It is obvious that only one reading can be correct, but as a matter of fact some variation was found on different parts of each slide. The Zeiss Objektmikrometer, for instance, at one point showed five spaces of the ocular micrometer (which is about the diameter of the average red cell) equal to 7.58 microns, at another point equal to 7.15 microns. A Leitz blood counting chamber at one point gave five spaces in the ocular equal to 7.16 microns, at another

⁶ Price-Jones, C. The Diurnal Variations in the Size of Red Blood Cells, *J. Path. & Bacteriol.* **23**: 371 (Dec.) 1920.

736 microns In order to overcome this variation ten readings were taken on each instrument and the results averaged We appreciate the fact that this does not correct this important source of error entirely, but we know of no more accurate method When we first calibrated

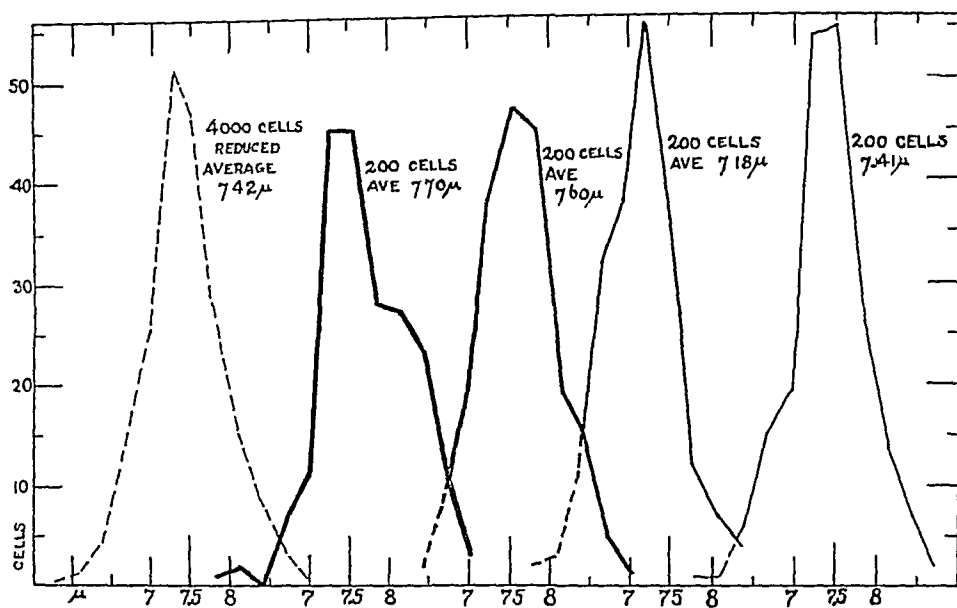


Fig 2—More extreme types of curve obtained from normal subjects

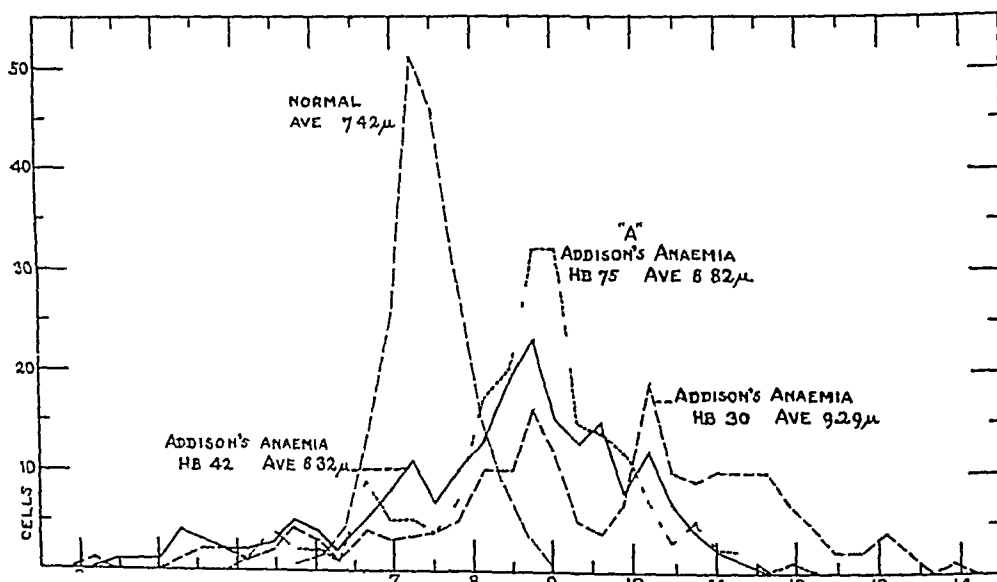


Fig 3—Three typical curves from cases of Addison's anemia compared with the normal, the great width of the curves, their irregular character, and that they lie largely to the right of the normal, making the average diameter larger than normal, should be noted

our ocular micrometer the average of all measurements showed five spaces to equal 7245 microns On finding that the average diameter of the red cells in our normal spreads was consistently larger than that of

Price-Jones, we repeated this calibration carefully and obtained practically identical results (7 252 microns). We have no doubt that the English workers appreciated the importance of accurate calibration and that their work was carefully done, but we record our curves as obtained.

In this work a Zeiss No. 4, $10\times$ ocular was used with a Zeiss $\frac{1}{12}$ oil immersion objective, N.A. 1.25, tube length 160 mm. We measured a few spreads by increasing the tube length until one space in the ocular micrometer equaled 1 micron. This would simplify to some extent the making of the curves, but the difficulty is to find the tube length at which one space equals 1 micron. With the instruments at hand we did not feel satisfied with this procedure, and abandoned it in favor of the procedure given above.

NORMAL CURVE

As noted above, we have determined the average normal diameter as 7.42 microns, Price-Jones determined it at 7.21 microns, Shackle and Hampson as 7.23 microns, and Capps as 7.65 microns. Our normal spreads were unselected as to age, sex and time of day. Price-Jones⁶ showed a very definite, although irregular diurnal variation in the average diameter (6), amounting at times to as much as 0.6 microns. Since in clinical medicine, however, spreads are ordinarily made at any time of day, we used the same procedure in this work.

Of more importance than the average diameter in a large group are the extreme types that may be regarded as normal. For this reason Figure 2 is given showing the variations encountered in normal spreads. It will be noted that the average diameters vary from 7.18 microns to 7.70 microns. These are simply the largest and smallest diameters found in our normals, and no doubt slightly larger and slightly smaller diameters will be found in normal persons on further search. But the character of the curve is to be noted as well as the position of the peak. The greatest difference between the largest and the smallest cell in any spread is 3.2 microns, i.e., there is little anisocytosis, the rise to the peak is abrupt, and the fall also, and the peak is well defined and occurs about at the average diameter. These are the characteristics we shall look for in examining the subsequent curves.

ADDISON'S ANEMIA

The most striking variation from the normal occurs, as one might expect in Addison's anemia (Fig. 3). Here there is a wide curve extending over 7 to 11 microns due to the great anisocytosis. There is no well defined peak, but occasionally two or three peaks occur, while the whole curve is pushed well to the right, i.e., the average diameter is increased. These characteristics occur in Addison's anemia regardless

of the stage of the disease. Figure 4 shows two curves from a case during remission. The curve more nearly normal was made when the hemoglobin was 90 per cent and the patient looked and felt perfectly well. At the same time there is considerable anisocytosis and the curve is pushed well to the right of the normal, making the average diameter 8.6 microns instead of 7.42 microns.

It is probably in the early differentiation of Addison's anemia from secondary anemia that this procedure will be of greatest value. Curve A in Figure 3 illustrates this point. This is from a woman of 62 years who complained of weakness, shortness of breath on exertion, and loss of appetite. If any pallor had developed recently, it was obscured by the fact that she had always been fair and had had white hair for years. Examination showed her well overweight, with a moderate arteriosclero-

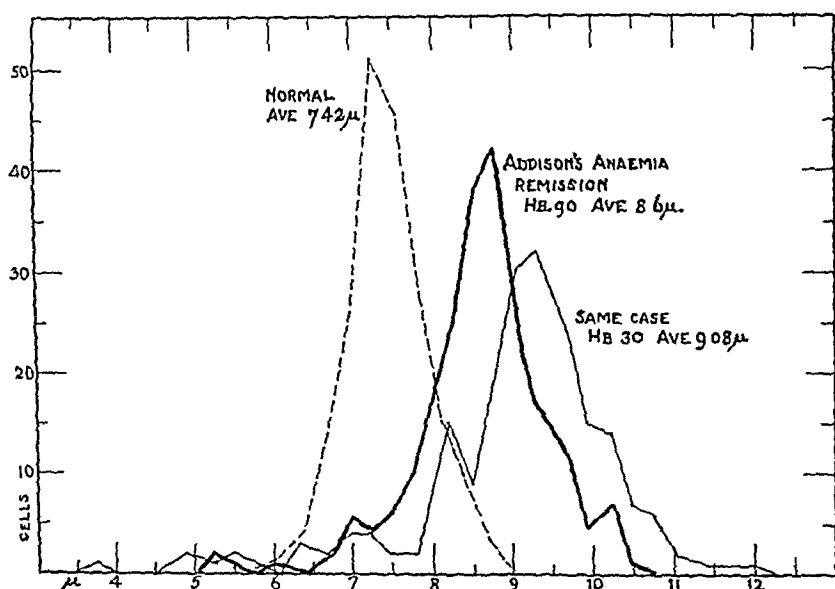


Fig 4—Addison's anemia in remissions the curve approaches normal, but still remains to the right of it

ris, hypertension, and cardiac enlargement. Her hemoglobin was 75 per cent, and spreads on ordinary examination looked practically normal. Nothing else of any consequence was found and we were about to make a diagnosis as indicated above, which would have explained the clinical picture satisfactorily, but decided to wait for the red cell curve. This showed a very striking variation in the size of the red cells, much more than one would expect from merely looking at the spreads, with the average diameter increased to 8.70 microns. Subsequently, complete absence of hydrochloric acid was found in the gastric juice, completing the evidence that this was undoubtedly an early case of Addison's anemia.

SECONDARY ANEMIA

In secondary anemia, whether due to infection, hemorrhage or new growth, the curves, while not so striking, are characteristic. The degree of anisocytosis is never so great as in Addison's anemia, and the average diameter is always either within normal limits or smaller than

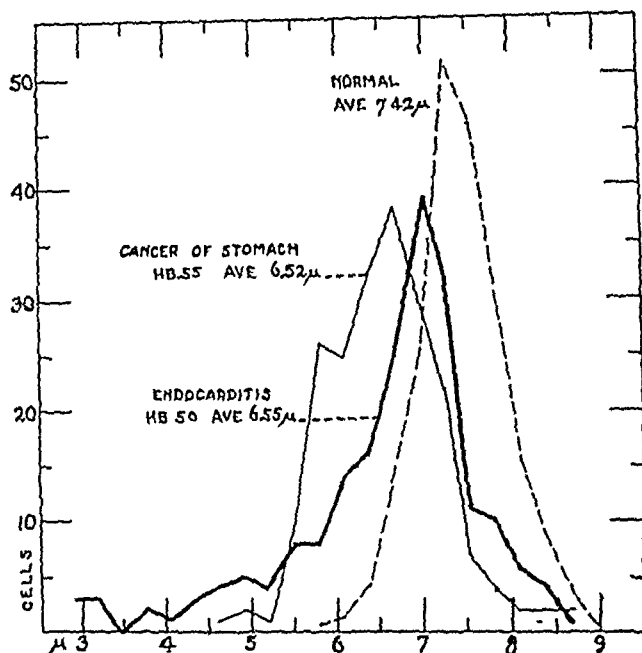


Fig 5—Two typical curves from secondary anemia, curves are pushed to left of normal, making average diameter smaller than normal

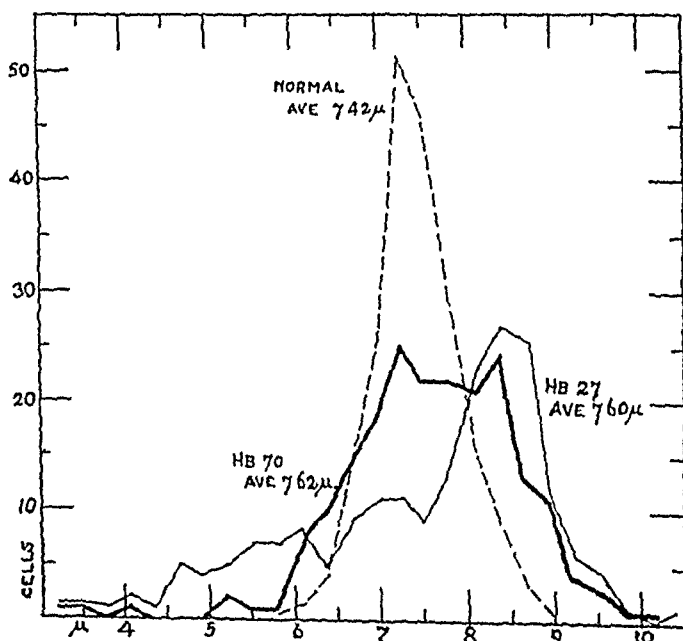


Fig 6—Two curves from same case of secondary anemia, while the hemoglobin in the one is very low the average diameter is within normal limits

normal. Two typical examples are shown in Figure 5. It is important to note that the decrease in the average diameter is not proportional to the decrease in the hemoglobin. A very severe anemia may show red

cells of normal or nearly normal diameter, but the important point is that the average diameter is never larger than the normal and never approaches even the smallest average diameter found in Addison's anemia

In this connection the most puzzling spreads which we encountered are those shown in Figure 6. They are from a man aged 46, who was brought to the hospital in coma. A history was obtained of at least two periods of severe anemia with fever in the last three years, and of loss of blood from hemorrhoids for the last four to six years. His hemoglobin on admission was 27 per cent, red cells totaled 1,224,000, white cells, 2,600. A temperature of from 100 to 103 degrees continued from three to four days, gradually subsiding. Later a test meal showed no hydrochloric acid in the gastric juice. His

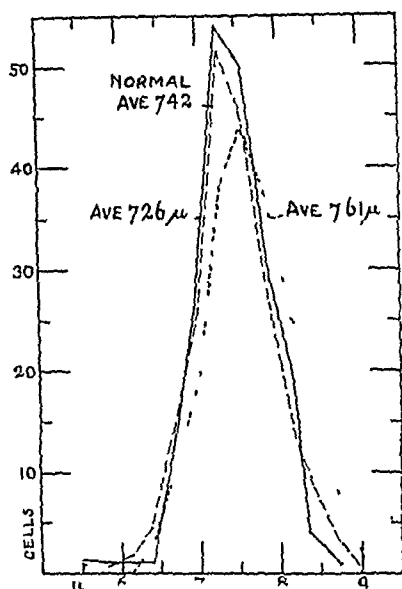


Fig 7—Curves from two cases of posterior lateral sclerosis with achylia but without anemia. These curves are essentially normal.

spreads showed considerable anisocytosis and poikilocytosis and the red cells looked about normal in color.

Here was conflicting evidence, the previous periods of anemia with fever, the achlorhydria, leukopenia and superficial examinations of the blood spreads indicating Addison's anemia, the history of prolonged hemorrhage indicating secondary anemia. Measurement of the red cells on several occasions, both while the anemia was at its worst and after the hemoglobin had been brought up to 70 per cent, showed the average diameters of the red cells to be 76 microns as shown in Figure 5. As this is about the upper limit of normal, it still remained a question whether this was a secondary anemia with a large normal diameter or an Addison's anemia with a diameter approaching normal. Were it

an Addison's anemia, however, there seems no doubt that at least when the hemoglobin was down to 27 per cent the average diameter would have been very definitely above normal and the degree of anisocytosis would have been much greater than it was. Whether the achlorhydria played any part in the production of the anemia is not entirely certain, but with the foregoing evidence it seems reasonable to suppose that the anemia was not of the Addisonian type but was secondary to the prolonged hemorrhage.

POSTERIOR LATERAL SCLEROSIS

Curves from two cases of posterior lateral sclerosis without anemia are shown in Figure 7 because of the close association of the disease with Addison's anemia. One of these patients had been under treatment for two years and was practically symptom free when the spreads were made. Both cases had an achlorhydria. The diameter curves are essentially normal. It will be interesting to see whether these patients develop any anemia in the future and whether the curves change.

CONCLUSIONS

A technic has been described for the measurement of the diameter of red blood cells and for the construction of diameter curves which is sufficiently accurate for clinical purposes and sufficiently simple to be used in a routine manner in examining any questionable blood spread.

The diameter curves are of value in the differentiation of Addison's anemia in all its stages and of secondary anemia.

RENAL FUNCTION IN PERSONS HAVING ONLY ONE KIDNEY *

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Man is born with at least twice as much kidney tissue as he requires. This fact is proved by the health of numerous persons who have sustained unilateral nephrectomy. Probably this wide margin of safety in the matter of surplus kidney substance has intimate relation to the difficulty of diagnosis of renal disease in its earlier or milder stages. So far as meeting the necessary demands for excretion it would seem to be an indifferent matter whether one kidney be removed or half the elements of each kidney be destroyed by disease.

For the purpose of the present study we are ignoring certain important features of nephritis, namely, evidence that the disease is constitutional rather than confined to an organ, that its tendency seems progressive, that connective tissue growth in healing within an organ produces of itself an extension of destruction of organic elements. But in the early stages of nephritis, at some time before symptoms of any kind are manifested, diagnosis depends to a considerable extent (though not exclusively) on signs of congestion in the kidney (albuminuria, casts, blood, etc.) or on evidence of impaired renal function. And it is because of the equivocal significance of albumin and casts in the urine that the modern tendency is to attach greater weight to tests of function.

For example, in the case of a man of 40, whose first intimation of unsound health comes as a rejection for life insurance on account of albuminuria, it is often very difficult to determine that albuminuria is due to nephritis, yet the probabilities are that it is.¹ The usual functional tests in such cases are normal and yield no data for diagnosis. The explanation for a normal response to tests in cases ultimately proved to be nephritis in after years may be that the extent of disease does not transgress the margin of safety, which is to say that the amount of normal tissue is adequate to meet the demands of our tests.

It has been suggested by Addis that some of the deficiencies in methods of diagnosis may be overcome by making use of functional tests that impose more strain than is the case with the usual tests. In

*From the first division, New York Hospital, and Cornell University Medical College.

¹ Insurable persons having a faint trace of albumin at the age of 40 or over have a death rate from nephritis which is ten times the normal, with a trace of albumin the death rate is about thirty times the normal, with large amounts of albumin and casts the rate is about fifty times the normal. Dublin, L. S. *Am J Hygiene* 1 301 (May) 1921.

principle, I think this idea is sound. And it is certainly true that in many cases of quite definite nephritis the response to such functional tests as phenolsulphonephthalein excretion, the concentration tests of Mosenthal and of Christian, the concentration of urea in the urine or in the blood, are all within the accepted zones of normal. The majority of these cases, however, fail to attain normal standards when tests that do impose considerable strain are used. Tests that come within the last category are the water excretion test of Volhard² and the concentration test as modified by Addis³. The defect in the water excretion test is due to the fact that renal function depends on the general circulation, hence on the integrity of the heart, but in the class of case in which the test is of most use, namely, in apparently healthy persons, this imperfection is not of serious moment. It is moreover supplemented by the concentration test. In the work of routine diagnosis these two tests have been found of great value. The experience afforded by more than 200 "early" cases suggests that while neither test alone is infallible the results of both together accord well with the later progress of the case.

With the restrictions and assumptions already mentioned, the question then arises as to what extent of injury of renal elements must occur before functional limitation can be detected. Specifically, is it possible to impose sufficient strain to disclose a limitation of functional response when the person has only one kidney, i. e., half the normal amount of functioning elements?

In selecting cases for test we have used only younger persons, since in any one over 40 there is the possibility of degenerations incident to age or vascular disease. These tests have in all cases been conducted within three weeks after the removal of the diseased kidney. This was done in order to exclude the possibility of the effect of hypertrophy of the remaining kidney, although it is generally believed that hypertrophy occurs only when one kidney is removed in early life. All of the cases reported had been studied with great care before operation and in none was there evidence of disease in the kidney that was left in situ. These tests included catheterization of the ureters and examination of the urine from each kidney, response to dye tests, etc. But since the most meticulous study cannot exclude tuberculosis of the kidney, the two cases of nontuberculous disease are most important (Cases 2 and 7). The blood chemistry and phenolsulphonephthalein excretion were normal in all these cases after operation.

First we shall consider the response to the concentration test³. During this test the patient was allowed no liquid for twenty-four

² Volhard. *Verhandl d 27 Cong f inn Med*, 1910. Volhard and Fahr. *Die Brightsche Nierenkrankheit*, Berlin, 1914.

³ Addis, T., and Drury, D. R. *J Biol Chem* 55: 105 (Feb.) 1923.

hours, no water, milk, soup, tea or coffee. He received the usual full "house" diet with these exceptions. The test commenced after breakfast on the first day and continued till breakfast the second day. Each voiding of urine was collected and tested for specific gravity.⁴ Especially significant were urines passed during the night and early morning at the end of the period. During the latter hours of this test the majority of normal persons void urine of a specific gravity of 1.030 or 1.034. With a small minority the concentration does not go higher than 1.024 or 1.026. In this series of patients with one kidney, only one responded to this test in a way that is undoubtedly normal (Case 7). The kidney was removed in this instance on account of an infection following an injury. The highest specific gravity in Cases 2 and 8 was 1.028, which may be normal.

Summary of Tests of Renal Function

Case	Diagnosis	Age	Highest Specific Gravity of Urine During Thirst Day	Volume of Urine in Four Hours After Ingestion of 1 500 C c of Water	Urea in One Hour's Urine				= X
					Urea in 100 C c Blood				
					First Hour	Second Hour	Third Hour	Average	
1	Tuberculous kidney	14	1 024	1,135	25 1	25 9	36 1	29	
2	Carcinoma	32	1 028	1,260	45	28 7	36 4	36 7	
3	Tuberculous kidney	29	1 022	945	28 5	29 7	25 1	27 7	
4	Tuberculous kidney	21	1 018	840	5 5	1 5	6 5	4 5	
4	Same case 5 months later				32 4	36 09	34 00	34 1	
5	Tuberculous kidney	31	1 026	1,745	36 21	22 87	27 81	28 89	
6	Tuberculous kidney	27	1 026	1,426	49 89	29 48	30 08	36 48	
7	Pus in kidney	27	1 030	1,660 (1 001)*	11 10	27 50		19	
8	Tuberculous kidney	14	1 028	1,330 (1 001)*	20 76	16 08	22 69	19 8	
9	Tuberculous kidney	21	1 027	1,014 (1 001)*	4 8	18 72	12 65	12 05	
9	Same case 2 weeks later				24 24	43 25	40 04	35 8	

* Specific gravity

The water excretion test² is conducted as follows. In the morning before breakfast the patient is given 1,500 c c of water which he drinks in the course of half an hour. The bladder is emptied before the test and all of the urine is collected thereafter for four hours. Normally more than a liter is excreted during this interval. In only two cases (Cases 3 and 4) was the result below a normal standard. It is perhaps surprising that the average is not lower in the series since it would seem that if in any relation the amount of tissue influences the result it would be apparent in this test. And in cases suspected of nephritis on account of albuminuria this test more often than any other one test is apt to indicate defective function.

⁴ The concentration of urea was estimated in many cases also, it was normal or above in all. On the concentration of urea especially McLean relies for diagnosis.

By studying the effects of changes in blood-urea concentration on the rate of urea excretion, Addis and Drury came to the conclusion that for special conditions the ratio $\frac{\text{Urea in one hour's urine}}{\text{Urea in 100 c.c. of blood}}$ is a constant for a wide range of blood-urea concentration

In applying this principle to clinical study two essential conditions are required (1) The kidney should be placed under circumstances that call for great activity in urea excretion, (2) there should be an absence of certain specific renal stimulants and depressants. The tests in this series have been done in the same manner as described by Addis.⁵ Since in our series the urea of the blood was normal, 30 gm of urea was the dose given in a liter of water. The hourly specimens of urine and the samples of blood were taken with care and analyzed for the urea content. Addis found the ratio in normal adults to average 50.4. Ratios between 34 and 40 are within a zone of doubtful interpretation, but ratios below 34 are abnormal. It is at once noted in our series that in no case does the function of one kidney as expressed in the ratio reach the level of average normal, namely 50. But if the average weight of the single kidney in these cases is 150 gm the theoretical ratio would be 25. This figure is considerably exceeded in four cases (Case 2, 36, Case 4, 34, Case 6, 36, Case 9, 35). In Cases 4 and 9 the ratio was determined again after an interval and the second result is of interest since it would indicate an enormous increase in the functional ability of the kidney. Two possible explanations suggest themselves for this improvement: the kidney might have hypertrophied, though this is not supposed to occur in adults, or at the time of the first observation the kidney might still have been affected by toxic depression—disease of one kidney is believed to induce this in the other. In both patients there had been a decided improvement in general health at the time of the second observation, possibly this was a factor. But the explanation for the fact must be left to conjecture.

When results of the tests of each patient are considered, not separately but together, as a whole from which some sort of general impression should be gained, the impression is, in two cases at least (Cases 2 and 6), that even these tests which impose severe strain have failed to indicate that the reserves of renal function have been fully determined, or, in other words, the decrease in renal tissue in a person with one kidney is only doubtfully revealed by these tests when the findings are compared with those of a normal subject. And the only test that shows consistently that there is a limitation of function is the ratio after giving urea.

⁵ Addis, T. Renal Function and Amount of Functioning Tissue, Arch Int Med 30 378-385 (Sept) 1922

If we are to depend to any extent on tests of function for the detection of renal disease (and at present there is no alternative) then it seems important to know whether any test can so tax the maximum effort of the organ that a loss of half its functioning tissue will be evident. This question is vital to methods of diagnosis as applied to the less advanced stages of renal degeneration.

In the somewhat artificial conditions imposed by this study it has been necessary to ignore considerations that are possibly of great importance clinically. While some diseased kidneys appear to be a result of discrete areas of degeneration, the neighboring structures remaining healthy, this is not invariably the case. It may happen that all portions of diseased kidney appear abnormal, the differences in various areas being in the degree of departure from the normal. Now it is impossible to predict or even to surmise to what extent even the most trivial change in a cell group will alter the function of these cells. And while the results of study of persons with only one kidney emphasize the limitations of diagnostic methods in the recognition of nephritis they also suggest that caution must be exercised in endeavors to estimate the amount of tissue still capable of function.

HYPERGLYCEMIA

I THE RELATIVE BLOOD VOLUMES IN DIABETES MELLITUS¹

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It has been known for a long time that diabetes mellitus is a disease characterized by a greater severity in young people than in persons at or beyond middle age. In young patients thirst, excessive diuresis and loss of weight are prominent symptoms, standing in sharp contrast to the relative or absolute comfort enjoyed by older people. Exsiccation, acidosis and coma occur frequently, while in older patients they are usually secondary to some definitely assignable cause, such as acute dietary indiscretion, the onset of an acute infection or cerebral hemorrhage. In an elderly patient it is usually only after some similar mismanagement or accident that the disease runs an acute course.

Polyuria and exsiccation are always associated with the severe form of the disease, but they are either inconspicuous symptoms or are absent altogether in the milder forms. It would seem then that diuresis and renal permeability must exert an important influence in determining the course. If so, all cases of severe diabetes, regardless of age, should show evidences of tissue and blood dehydration. All cases of diabetes in young persons should show more evidence of tissue and blood dehydration than occurs in the elderly. Elderly patients should be expected to exhibit scarcely any evidence of dehydration, perhaps none at all in the absence of polyuria. I have tried to show that these suppositions are correct.

The presence or absence of tissue exsiccation has been estimated by clinical observations of the mucous membranes, skin and subcutaneous tissues. Anhydremia and hydremia have been determined by measuring the relative volumes occupied by serum and by erythrocytes in serial samples of defibrinated blood. This has been done by means of the electrical conductivity measurements of the whole blood and of its serum. From these the relative volumes were calculated by means of the relation developed by Stewart¹. All conductivity measurements have been corrected to 5 degrees C, and are reported as specific conductivities, that is, $K \times 10^4$ at 5 degrees C. The erythrocyte counts were all made in duplicate, with the same pipet and counting chamber. All counts that

¹ From the department of medicine of Lakeside Hospital and Western Reserve University School of Medicine

¹ Stewart, G. N. The Relative Volume or Weight of Corpuscles and Plasma in Blood, *J. Physiol.* **24** 356 (July) 1899

showed a variation of more than 250,000 were discarded. In each instance the average of the two counts was used for the final figure. These were all made on defibrinated blood. Blood sugar determinations were made by the method of Folin and Wu.²

It must be understood that all statements pertaining to the relative blood volumes refer to conditions existing in the circulating blood of an

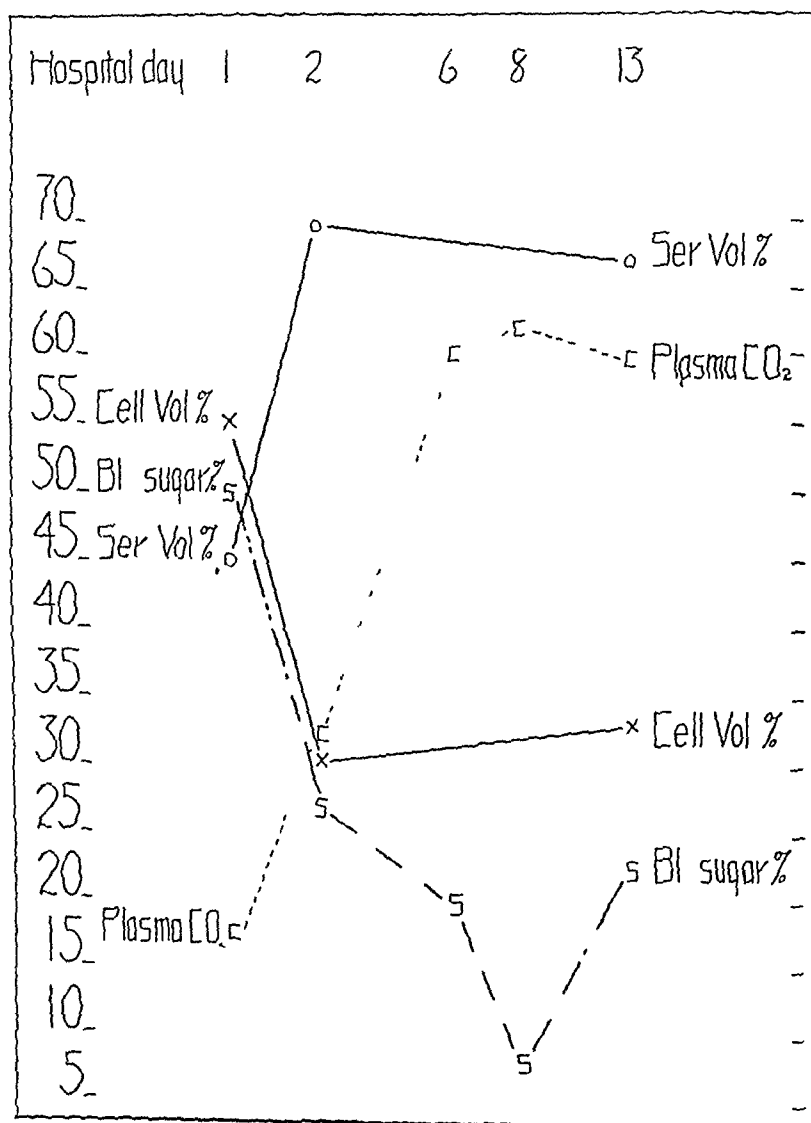


Fig 1 (Case 33) —Diabetic coma in young patient with recovery after insulin treatment, severe degree of blood concentration on day of admission, effects of insulin — increase in relative volume of serum, increase in plasma carbon dioxide and decrease in blood sugar

antecubital vein. The greatest possible care was exercised both in collecting and in defibrinating the samples.

² Folin, O., and Wu, H. System of Blood Analysis, J Biol Chem 38 81 (May) 1919

CHANGES IN YOUNG PATIENTS

In young patients not under treatment it has been found that hyperglycemia invariably produces an increased concentration of the blood. The initial blood sample, taken on admission to the hospital, has always been more concentrated than any succeeding sample. Whenever erythrocyte counts have been made, they have always been higher in the first sample. Unless the patient is anemic, the relative volume of serum has always been less than the normal in the first sample, increasing toward the normal as the patient becomes more and more under the influence of dietetic management and insulin treatment. Then, in some

TABLE 1—*Blood Concentration Changes in Young Diabetic Patients*

Case	Date	K $\times 10^4$ at 5 Degrees C		C c Serum in 100 C c Whole Blood	Changes in Serum, Percentage by Volume	Blood Sugar, per Cent	Red Blood Cells in Millions, per C Mm	Remarks
		Blood	Serum					
3	1/11/24	30.0	77.2	55.96		0.33		
	1/13/24	32.0	78.2	58.11	+ 2.15	0.15		
5	2/ 6/24	24.6	72.4	50.75		0.37		
	2/11/24	26.2	73.7	52.54	+ 1.79	0.24		
	3/ 7/24	40.1	72.3	74.27	+23.52	0.23		
	3/11/24	32.6	73.0	63.15	+12.40	0.16		
8	3/ 3/24	26.3	74.6	52.06		0.30		
	3/18/24	28.0	74.1	55.19	+ 3.13	0.19		
9	2/ 6/24	24.8	69.4	53.31		0.37		
	2/ 9/24	27.4	76.5	52.51	- 0.80	0.30		
12	3/10/24	27.5	74.4	54.15		0.21		
	3/12/24	28.2	73.2	56.56	+ 2.41	0.33		
16	9/28/23	22.3	65.6	51.66		0.30		
17	10/14/23	26.3	73.0	53.28		0.34		
	6/18/24	25.6	70.4	53.94	- 0.66	0.46	5.840	
20	5/23/24	26.5	73.0	53.61		0.43	5.350	Coma, death
23	6/ 8/24	30.7	76.1	57.88		0.20	4.800	
	6/13/24	33.4	73.2	64.24	+ 6.36	0.18	3.900	
	6/17/24	33.5	73.2	64.22	+ 6.34	0.38	4.300	
28	6/10/24	30.0	74.5	57.98		0.24	5.210	
	6/17/24	32.7	74.5	62.03	+ 4.05	0.22	4.840	
33	9/11/24	19.3	66.7	44.67		0.50	6.450	Coma
	9/12/24	37.8	74.0	69.46	+24.79	0.28	3.545	Recovery
	9/23/24	34.8	72.5	66.80	+22.13	0.23	5.155	
34	9/23/24	50.6	72.8	84.77		0.08	2.445	Secondary anemia
	9/26/24	48.6	70.8	85.97	+ 1.20	0.38	2.855	Coma, death
36	11/20/24	21.1	76.6	42.2		0.38	7.310	Coma, death

cases, the gross viscosity of the blood is visibly decreased. These changes are well shown in the data from the cases presented in Table 1 and also in Figure 1.

Excessive diuresis was present in each case. Every patient was markedly underweight and, despite increased thirst and fluid intake, presented dry mucous membranes and a harsh, dry skin.

CHANGES IN ARTERIOSCLEROTIC PATIENTS WITH ACUTE
EXACERBATIONS OF DIABETES

The data in Table 2 represent the same findings in all arterio-sclerotic diabetic patients who were admitted to the hospital because of

acute dietary indiscretions, acute infections or cerebral hemorrhage or thrombosis. Here again, the conditions on admission indicated increased blood concentration. If recovery occurred under treatment the blood concentration diminished, while if treatment was unsuccessful and the patient died, the blood became even more concentrated. Figure 2 illustrates a recovery.

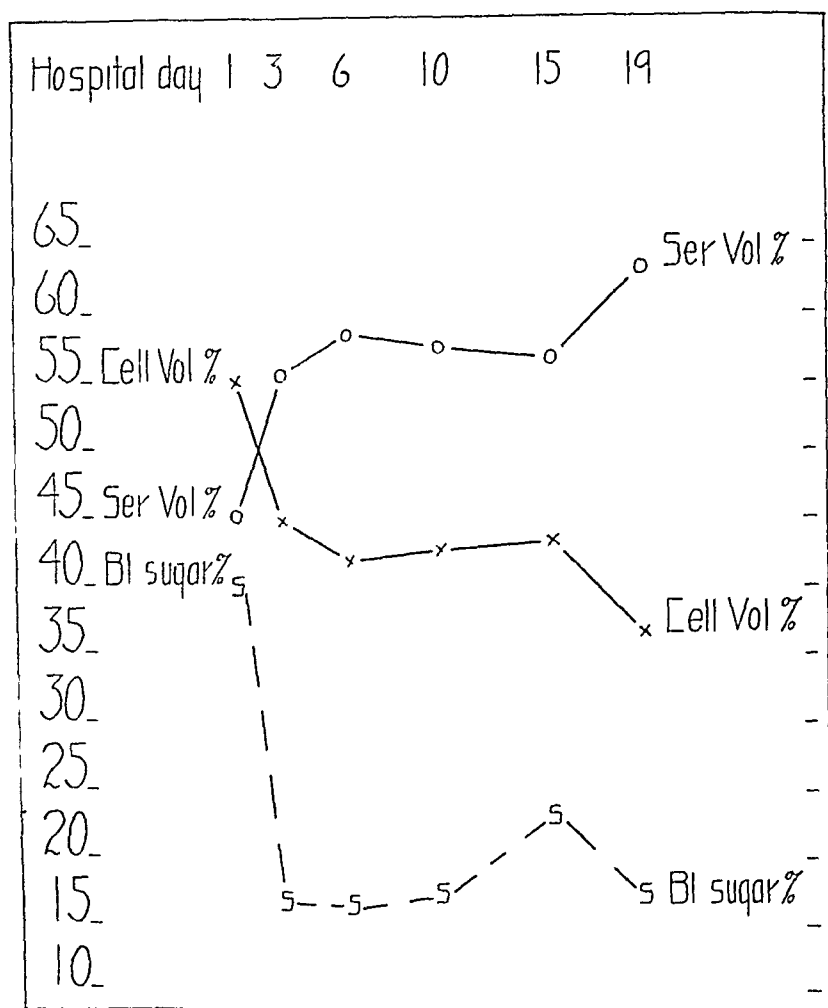


Fig 2 (Case 15) —Acute exacerbation of diabetes in patient with advanced arteriosclerosis, extreme reduction of relative volume of serum on first day and prompt increase following insulin administration

Here, also, increased diuresis was a prominent symptom. Emaciation was not observed but in every instance the mucous membranes and skin showed marked evidences of tissue dehydration.

CHANGES IN ARTERIOSCLEROTIC DIABETIC PATIENTS

In this group, represented by the data in Table 3, the initial blood samples do not show any evidence of increased blood concentration. On the contrary the majority of them indicate a blood dilution, and

in the remainder the water content of the blood appears not to have been disturbed. In these patients the response to dietary and insulin treatment is a return from the initial dilution back toward the normal condition, an *increase* in blood concentration (Fig 3). In some instances the gross viscosity of the blood is visibly increased after the hyperglycemia has disappeared.

These patients did not suffer from thirst and polyuria, and there was only slight clinical evidence of tissue dehydration. In each case examination of the accessible peripheral arteries and of the ocular fundi gave unmistakable evidence of advanced arteriosclerosis. The latter also is true of the cases in Table 2.

TABLE 2—*Blood Concentration Changes in Arteriosclerotic Diabetic Patients Presenting Acute Exacerbations*

Case	Date	K $\times 10^4$ at 5 Degrees C		C c Serum in 100 C c Whole Blood	Changes in Serum, Percentage by Volume	Blood Sugar, per Cent	Red Blood Cells in Millions, per C Mm	Remarks
		Blood	Serum					
2	12/14/23	23.1	72.1	48.35	.	0.60		
	12/15/23	34.4	81.6	58.84	+10.19	0.20		Coma
	1/13/24	35.2	74.4	65.67	+17.32	0.12		Recovery
6	2/22/24	32.2	72.0	63.41		0.56		
	3/7/24	38.5	71.0	73.47	+10.06	0.37		Gangrene of foot
	3/11/24	40.3	73.0	73.0	+9.59	0.51		
11	3/3/24	26.0	67.2	57.28		0.32		Coma
	3/6/24	23.8	67.5	52.96	-4.32	0.27		Death
14	4/20/24	26.6	71.9	54.53		0.35	5.330	
	4/21/24	34.5	76.9	62.53	+8.05		4.215	Coma
	4/22/24	28.6	74.3	55.88	+1.35	0.60	5.355	Death
15	5/5/24	21.6	73.5	44.67		0.40	7.350	
	5/7/24	31.0	80.1	55.48	+10.81	0.17	5.810	Cerebral thrombosis
	5/10/24	31.9	77.2	58.70	+14.03	0.16	5.570	
	5/14/24	32.4	80.0	57.40	+12.73	0.17	5.120	Coma
	5/19/24	30.0	76.1	56.84	+12.17	0.24	5.700	Recovery
	5/23/24	37.9	81.4	63.83	+19.16	0.17	5.920	
19	12/9/23	26.4	75.9	51.34		0.25		
21	5/20/24	23.9	73.9	48.56		0.37	6.420	
	6/11/24	26.3	73.2	52.45	+3.89	0.20	5.450	Food indiscretion
	6/18/24	28.3	74.8	55.09	+6.53	0.18	5.970	
1	11/23/23	34.4	74.5	64.38		0.53	4.700	Gangrene of foot
	11/26/23	41.3	77.2	70.93	+6.55	0.15		Acute cellulitis
	12/15/23	43.7	79.1	71.98	+7.60	0.14	3.800	Secondary anemia

That actual increase or decrease in the total number of erythrocytes was not a factor in this dilution is shown by the fact that when water balance had been restored, and the bloods remained at equilibrium so far as the relative blood volumes were concerned, the erythrocyte counts and the cell volume percentages corresponded to the normal average ratio found for all treated diabetic patients, whether young or old. This is 5,000,000 erythrocytes per cubic millimeter for 38 per cent relative cell volume.

CHANGES IN DIABETIC PATIENTS LONG UNDER INSULIN TREATMENT

When diabetic patients have been under constant dietary and insulin treatment for six months or longer, departures from the normal blood

concentrations depend entirely on the blood sugar level at the time at which samples are taken. If the food intake and insulin dosage are well balanced, so that the blood sugar does not rise much above 0.15 per cent, excessive thirst and diuresis are absent and the relative blood

TABLE 3—*Blood Concentration Changes in Arteriosclerotic Diabetic Patients*

Case	Date	K $\times 10^4$ at 5 Degrees C		C c Serum in 100 C c Whole Blood	Changes in Serum, Percentage by Volume	Blood Sugar, per Cent	Red Blood Cells in Millions, per C Mm	Remarks
		Blood	Serum					
4	1/11/24	32.8	78.2	59.23		0.35		
	1/13/24	32.8	78.1	59.30	+0.07	0.30		
	1/23/24	33.2	77.8	60.08	+0.78	0.16		
7	2/22/24	41.4	77.1	71.21		0.37		
	3/ 8/24	38.6	77.1	67.78	-3.43	0.15		
	3/13/24	33.5	74.4	63.23	-7.98	0.20		
10a	3/ 3/24	31.5	76.0	59.05		0.46		First admission
	3/ 7/24	26.6	73.0	53.71	-5.34	0.21		
	3/11/24	24.8	73.6	50.28	-8.77	0.19		
10b	4/30/24	30.6	76.8	57.09	-1.96	0.33	5.595	Readmission
	5/10/24	31.7	78.8	57.22	-1.83	0.18	5.700	
13	4/18/24	32.6	75.7	60.81		0.30	4.995	
	4/22/24	31.7	76.1	59.19	-1.62	0.27	6.195	Terminal pneumonia
	4/30/24	33.0	75.0	62.0	+1.19	0.17	5.090	
	7/10/24	29.3	77.9	54.40	-6.41	0.26	5.920	Death
22	6/ 8/24	37.5	80.5	63.62		0.07	5.440	
	6/13/24	36.9	75.6	66.91	+3.35	0.15	4.670	
	6/17/24	33.0	76.1	61.11	-2.51	0.21	4.980	
26	6/10/24	29.5	74.8	56.95		0.17	6.740	
	6/17/24	27.5	71.5	56.25	-0.07	0.22	6.080	

TABLE 4—*Blood Concentration Changes in Diabetic Patients Long Under Treatment*

Case	Date	K $\times 10^4$ at 5 Degrees C		C c Serum in 100 C c Whole Blood	Changes in Serum, Percentage by Volume	Blood Sugar, per Cent	Red Blood Cells in Millions, per C Mm	Remarks
		Blood	Serum					
18	12/ 9/23	29.7	78.0	54.83		0.14		No arteriosclerosis
24	6/10/24	30.7	72.2	60.92		0.14	6.730	
	6/17/24	32.2	73.5	61.90	+1.07	0.12	5.450	Child
25	6/10/24	32.3	74.8	61.09		0.13	6.170	No arteriosclerosis
	6/18/24	33.2	74.8	62.52	+1.43	0.16	5.020	
29	6/10/24	31.4	74.5	60.17		0.16	5.190	Slight arteriosclerosis
	6/17/24	33.2	74.9	62.44	+2.27	0.14	4.840	
30	6/11/24	29.4	72.2	58.86		0.26	5.360	No arteriosclerosis
	6/18/24	28.9	72.2	58.77	-0.09	0.24	4.720	
37	11/26/24	28.8	77.1	54.18		0.21	6.160	Young adult
		28.9	76.7	54.70	+0.52	0.23	5.560	
		27.9	75.4	54.19	+0.01	0.21	5.740	
38	12/ 4/24	29.4	73.6	57.68		0.25	5.210	Young adult
		32.6	75.0	61.41	+3.73	0.25	5.710	
		29.6	72.8	58.76	+1.08	0.30	5.220	
		29.0	74.7	56.36	-1.27	0.23	5.950	
J	2/ 4/25	30.0	71.4	60.5		0.27	4.955	Young adult
		29.5	70.9	60.1	-0.38	0.26	4.630	
		28.6	70.0	59.6	-0.90	0.21	4.700	
		29.2	71.4	59.4	-1.10	0.23	4.485	

volumes are within normal limits. In Table 4, Cases 24, 25 and 29 show the results in this well balanced state.

If there is a departure from the normal blood concentration, the direction of the variation seems to depend on the presence or absence

of arteriosclerosis, with normal arteries there is an increased concentration, whereas in the presence of arteriosclerosis a blood dilution occurs. In other words, during periods of unsatisfactory management there is a return from the satisfactory condition mentioned in the foregoing to that which classified the patient on admission. Cases 30, 37, 38 and 39 in the same table represent young diabetic patients in whom treatment was not satisfactory at the time. In each instance the blood is

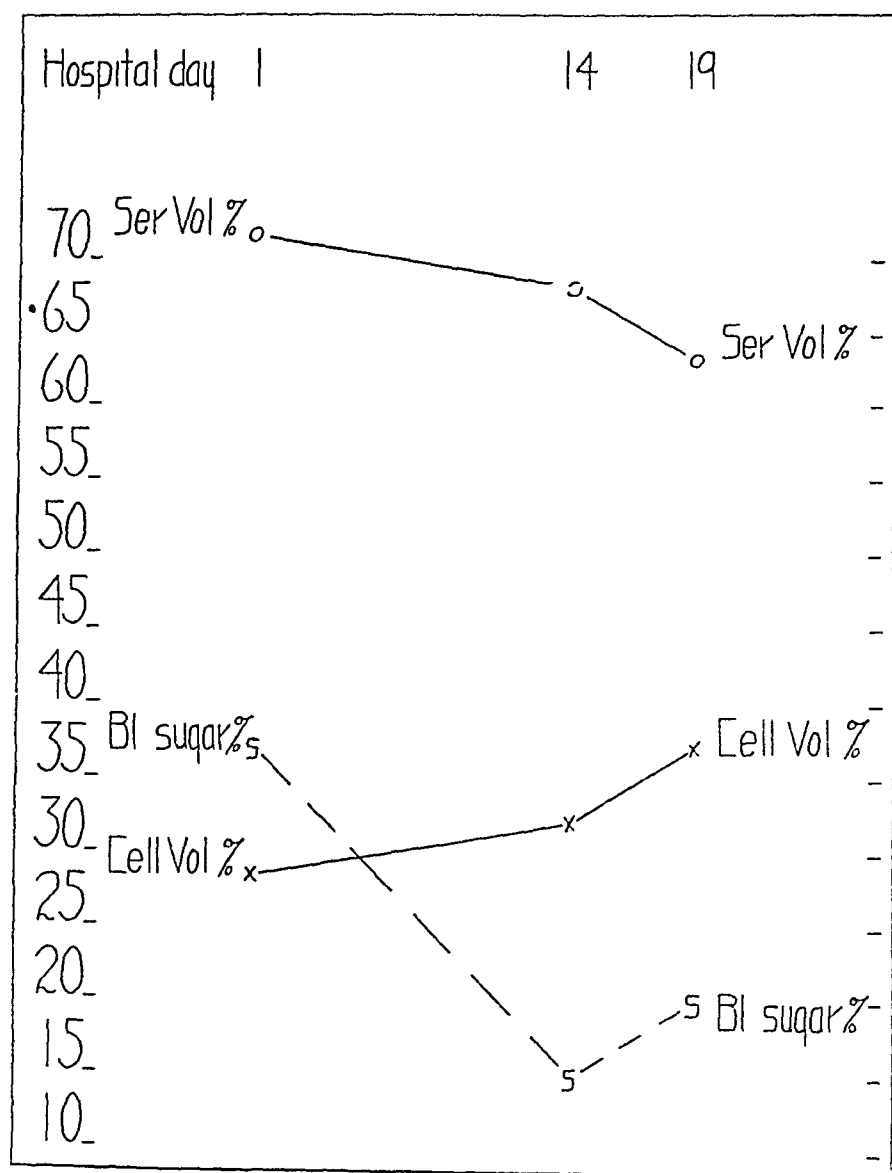


Fig 3 (Case 7) —Diabetes with advanced arteriosclerosis, gradual onset of symptoms, initial blood dilution, in this case insulin treatment caused a blood concentration

more concentrated than the average normal. Case 10 in Table 3 shows what happened to an arteriosclerotic diabetic patient during her absence from the hospital. She became careless about her diet, developed a hyperglycemia and again diluted her blood almost to the same extent found on her first admission.

In the event of mismanagement or infection all young diabetic patients will be reclassified into Group 1, and the amount of increased blood concentration will depend on the degree of hyperglycemia that results and the period of time during which the higher level is attained, the more rapid the increment in blood sugar the greater the increase in blood concentration. In the case of arteriosclerotic diabetic patients, if the increment in blood sugar is rapid they will be reclassified into Group 2, but if it is slow, they will fall into Group 3.

Thus the findings in this series of cases of diabetic patients support the hypothesis presented in the second paragraph, and suggest the following explanation. An increase in the concentration of glucose in the blood above the average upper normal limit causes water to be drawn into the blood stream, producing a state of hydremia. If the patient has normal kidneys and blood vessels, glycosuria and polyuria result and lead to tissue exsiccation, then to relative anhydremia or blood concentration, and eventually to severe water loss and acidosis. On the other hand, if there is much arteriosclerosis, even though the blood sugar slowly increases to 0.30 per cent or 0.35 per cent, only a little glycosuria or polyuria occurs or even none at all, and the result is simply a relative hydiemia or dilution of the blood. In young people the increase in fluid intake cannot keep pace with the increased output, but in arteriosclerotic diabetic patients, even though there may be some generalized tissue exsiccation, the fluid intake is sufficient to prevent blood dehydration. However, as I have already shown, if the rise in blood sugar occurs rapidly, all patients, regardless of age or arteriosclerosis, speedily become exsiccated.

CONCLUSIONS

Diabetes mellitus produces marked changes in water distribution and in the total water balance of the body.

The degree of these changes is modified by the magnitude of the hyperglycemia and by the rate of onset.

Their character and direction is modified by vascular disease and renal permeability.

Reduction of hyperglycemia (or the action of insulin) may produce either an increase or a decrease in the concentration and viscosity of the blood. The result in any given case depends on the antecedent water content.

Book Reviews

OPOTHERAPIE ENDOCRINIENNE LES BASES PHYSIOLOGIQUES, LES SYNDROMES, LA POSOLOGIE DE L'OPOTHERAPIE PAR LES GLANDES A SECRETIONS INTERNES By GUY LAROCHE, Medecin des Hopitaux de Paris Pp 256, illustrated Paris Masson et Cie, 1925

This volume deals with the general principles of organotherapy. It contains one chapter on the chemistry and the processes of production of extracts of animal tissues, one long chapter on the thyroid and thyroid disorders and their treatment, a brief chapter on parathyroids and the thymus, an exhaustive chapter on suprarenal disorders, and shorter chapters on the hypophysis, the testes and ovarian disorders and their treatment. The chapter on the pancreas deals almost exclusively with insulin, and there is a short but sane chapter on the organotherapy of so-called pluriglandular syndromes.

The volume as a whole gives evidence that the author is familiar with the important literature in this field, although reference to this literature is meager. The author further shows good judgment in most cases when dealing with the analysis and conclusions in fields in which great uncertainty still exists both as to etiology and therapy. For example, in the chapter on the so-called pluriglandular disorders and their treatment, he wisely rejects the claim that it is good therapy to give a mixture of glandular extracts because the body will pick out what it needs for its rehabilitation and reject the others. The book may be read with profit by physicians.

OPOTHERAPIE CLINIQUE ORGANOTHERAPIE-FORMULAIRE. By MARCEL LAEMMER, Pp 151, brief index Paris Masson et Cie, 1925

This volume is essentially a book of formulas of organotherapeutic preparations as used and endorsed by the author, and possibly by other physicians, in many types of disorders. The author lists organotherapeutic products not only from the recognized endocrine glands but also from organs and tissues like the pancreas, the liver, the salivary glands, the intestines, the stomach, the cerebrum, the heart, the lungs, the bones, the muscles, the placenta, the bone marrow, the lymph glands and the mammary glands. All of these, according to the author, have been used with therapeutic success in human disease.

He says, "It is sufficient to have employed with therapeutic success extracts of the heart, the cerebrum or the lung to establish their real therapeutic value." "Such clinical facts exist and let us accept them with joy, even though the way in which these results are obtained still remain obscure." But so far as the reliable clinical literature today is concerned, the success of such therapies is achieved on the principle of post hoc reasoning.

On Page 36 the author states as proved facts that the pancreatic secretion inhibits suprarenal activity, that the thyroid hormone inhibits the pancreas, that the thymic hormone inhibits suprarenal activity, and that the mammary hormone inhibits the ovaries. Without much fundamental discussion the author on Page 72, gives several formulas of alleged organotherapeutics of value in the treatment of epilepsy. One of these runs: dessicated thyroid, dessicated parathyroid, dessicated ovary, with calcium carbonate.

On Page 102 the following formula is given for the treatment of constipation: dessicated duodenal mucosa, dessicated ox bile, and powdered agar-agar. For chronic nephritis and uremia he gives a mixture of dessicated thyroid, dessicated liver and dessicated heart (Page 117).

On Page 123 we are told that one may use successfully thyroid extract, suprarenal extract, ovarian extract and parathyroid extract for middle ear sup-puration. On Page 132 the following formula is given for the treatment of inoperable cancer: dessicated thyroid, dessicated bile, dessicated liver, bone marrow, with manganese sulphate.

The volume seems totally unreliable as a guide for physicians. The book might be understandable as a satire on the credulities and follies of ancient and modern organotherapy, but taken as a serious contribution to biology and medicine, one would have to look far for a monograph on organotherapy equally credulous and unreliable.

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